Doctor of Medicine (MD) Thesis

Impact of 5-HT$_3$ receptor blockade on the subjective and behavioural effects of drugs of abuse in humans

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1. SUMMARY

Substance abuse is a major health problem world-wide. Whatever the treatment goal, be it abstinence, 'controlled' use, or relapse prevention, the outcome from both pharmacological and non-biological treatments remains disappointing. In recent years, animal experiments have suggested that the reinforcing properties of drugs of abuse are critical to drug-seeking behaviour. Dopaminergic fibres running from the ventral tegmental area to the nucleus accumbens play a central role in the mediation of alcohol-induced reinforcement. Notably, 5-hydroxy-tryptamine$_3$ (5-HT$_3$) receptor antagonists, which antagonize dopaminergic activity in the nucleus accumbens, have been shown to inhibit ethanol consumption in a free-choice paradigm, and to attenuate the reinforcing effects of amphetamine in some animal models. Obviously, the demonstration of a similar effect in humans would have important clinical implications for the treatment of substance abuse. In humans, however, reinforcement is difficult to measure directly. Nevertheless, the pleasurable subjective effects of drugs of abuse are important behavioural correlates of the reinforcement process. In the present thesis I investigated the effects of 5-HT$_3$ receptor antagonists on the subjective positive effects of alcohol and amphetamine. In addition, the independent and interactive effects of a 5-HT$_3$ antagonist and amphetamine on hunger, caloric intake and macronutrient selection, and cognitive performance was studied. The 5-HT$_3$ antagonist, ondansetron, reliably reduced the pleasurable subjective effects and the desire to drink alcohol. In addition, evidence was provided to

demonstrate that ondansetron did not simply reduce the absorption of alcohol, as it has the ability to slow gut motility. Thus, these experiments are the first clear evidence that 5-HT$_3$ antagonists can decrease the reinforcing properties of alcohol in humans. It is, however, important to point out that alcohol consumption was not measured directly, and it is possible, as has been demonstrated with other drugs such as cocaine, that blockade of reinforcement can, paradoxically, increase consumption and the subject works harder to obtain the drug. While repeated dosing with ondansetron also attenuated some positive subjective effects and the anorexic properties of d-amphetamine; in contrast, a single dose of the second generation 5-HT$_3$ antagonist, GR 68755, was without effect on mood, hunger, satiety, and food intake. This suggests that, as in recent electrophysiological studies, 5-HT$_3$ antagonists may only influence the reinforcing properties of drugs which indirectly facilitate dopamine neurotransmission via 5-HT activity at excitatory pre-synaptic 5-HT$_3$ receptors, and are not effective against drugs which, simply, cause the direct release of dopamine from nerve terminals and do not increase dopamine cell firing transynaptically. It is, therefore, possible that the successful attenuation of amphetamine-induced subjective state and hunger by ondansetron was due to kinetic effects, which presumably became more manifest with repeated rather than single dosing, possible post-synaptic effects at 5-HT$_3$ receptors or interactions with other neurotransmitter systems which are, at present, poorly understood, or chance. Further studies are needed, however, to investigate the effectiveness of a pharmacological range of doses of 5-HT$_3$ antagonists on amphetamine-induced behaviour. GR 68755

was without effect on the natural increase in subjective feelings of hunger over time following an overnight fast, and the pattern of caloric intake and macronutrient selection was similar to placebo. D-amphetamine reliably reduced hunger, and this was shown using a test meal to lead to a global decrease in caloric intake and no macronutrient was selectively spared or consumed. Interestingly, both GR 68755 and amphetamine alone improved cognitive performance but this effect was not additive suggesting different neurotransmitter systems may be involved. In future, I intend to extend this human laboratory work by uncovering the therapeutic range of ondansetron with respect to decreasing the positive subjective effects including the desire to drink in alcohol abusers, and to investigate what impact this has on alcohol consumption. If these experiments are successful a clinical trial would be warranted.

2. An overview of alcohol and amphetamine related problems

2.1 Introduction

Alcohol misuse is a major and growing health problem world-wide (Armyr et al., 1982; Hein and Pittman, 1989; Karrenbrock, 1990; Helzer and Canino, 1992). While modest drinking is socially accepted in most cultures, there has been, in recent years, growing public awareness about the effects of excessive alcohol consumption on both physical and mental health, and safety. In Britain, up to one fifth of all males admitted to General Hospitals may have alcohol problems (Jarman and Kellet, 1979). In addition, two illustrative clinical surveys have suggested that between 10% and 15% of psychiatric hospital in-patients are alcohol abusers (Glass and Jackson, 1988; Johnson, 1990 respectively). One third of drivers killed in road traffic accidents have blood alcohol levels above the legal limit (Department of the Environment, 1976), and excessive drinking is highly correlated with domestic violence (Orford, 1979; Smith, 1989), public order offences (Tuck, 1989), and homicide (Gillies, 1976; Gudjonsson and Petursson, 1990). It is, therefore, of concern that excessive drinking may be on the increase: for example, the Scottish Council on Alcohol (1988) reported that alcohol consumption per head of population rose from 6.9 litres in 1975 to 9.1 litres in 1985. In the United States the situation is equally problematic, with an increasingly greater proportion of the health care budget being devoted to the management of alcohol-related problems: from $136 billion in 1990 to an estimated $150 billion in 1995 (Secretary of Health and Human Services, 1990).

In Britain, unlike the United States, there is no nation-wide system, such as the Drug Abuse Warning Network (DAWN), for tracking morbidity and mortality due to drug abuse. Nevertheless, the media having abstracted without caution from the United States situation, have excited the public with shocking stories attributable to the use of stimulants drugs; notably, amphetamine and amphetamine-like substances, and *"crack"* cocaine. In particular, stimulants have been associated with the spread of the human immunodeficiency virus (HIV), gang violence, and the sudden death of teenagers at *"rave"* parties where the use of the amphetamine-like substance 3,4-methylenedioxymethamphetamine (MDMA or *ecstasy*) is almost endemic (Royal College of Psychiatrists, 1987). It is now widely accepted that amphetamine misuse occupies a dominant position in the UK drug scene (Drugs Branch Inspectorate, 1987). In 1988, Stimson and co-workers reported that, despite striking regional variations, about 15% of attenders at needle exchange programmes misused amphetamines; of these, more than half were estimated to have injected within four weeks of presenting. It has been suggested that the increased propensity of amphetamine misusers to inject with friends (Klee, 1992) and to be sexually active afterwards (Andreason et al, 1991; Kall and Olin, 1991) may lead to greater risk of HIV seropositivity among amphetamine users compared with those who inject other drugs (Harris et al, 1991).

In the United States, amphetamine abuse reached epidemic levels in the 1970's, and then subsided - over 10 billion 5 mg tablets were produced (Grinspoon and Hedbloom, 1975). Recently, there has been an outbreak of

d-amphetamine abuse on the Pacific Coast, Hawaii, and in some communities stateside (U.S. National Institute on Drug Abuse {NIDA} 1989 and 1990) but fears of a national crisis have, as yet, failed to materialize (CHO, 1990).

2.2. Treatment Programmes for alcohol and amphetamine

The last twenty years has seen changes in the treatment of alcoholism. Intensive in-patient programmes, for all but the most intractable of cases, rapidly fell out of favour following criticism by the WHO Expert Committee (1980) of its expense and lack of efficacy at preventing relapse. Emphasis shifted towards the *"traditional"* method which stressed psychotherapy, education, and social manipulation. Recently, there has been an expansion in programmes incorporating the Alcoholics Anonymous (AA) ideology - the best known of these being the Minnesota model (Cook, 1988). Partly as a reaction to this AA model, which has aroused much controversy, a cognitive-behavioural approach (i.e. training the alcoholic in self-control techniques) has become increasingly popular (Sanchez-Craig, 1990). In the UK early identification of the *problem drinker* (i.e. an individual whose excessive alcohol consumption gives rise to medical, psychological, or social problems) by the family practitioner (Pollack, 1989) using a screening questionnaire such as the *'CAGE'* (Roche et al., 1984) followed by treatment with brief counselling (Edwards, 1977) and specialist support if necessary has been shown to be of benefit (Drummond et al., 1990). In the *'CAGE'* questionnaire, individuals are asked: "Have you ever felt you should *cut* down on your drinking?" "Have people *annoyed* you by criticizing your drinking?" "Have

you ever felt bad or *guilty* about your drinking?" "Have you ever had a drink first thing in the morning to steady your nerves (*eye-opener*)?" If two or more positive answers are taken to indicate problem drinking, most *problem drinkers* are identified; nevertheless, specificity is not as good with a false-positive rate of about 25% (Mayfield et al, 1974). Treatment is directed at motivating (Prochaska and Di Clemente, 1986) the individual to decrease or cease alcohol consumption altogether.

Irrespective of the type of treatment programme, and each has its advocates, response to treatment remains poor. The influential Rand Report (Armor et al., 1976) which compared 45 treatment centres in the USA showed that only a quarter of alcohol abusers remained abstinent after six months, although the majority had reduced their drinking. Unfortunately, the findings of more recent studies have been hard to interpret for three reasons. Firstly, advocates of each particular programme have been most successful at demonstrating its greater efficacy over other methods in their own clinics. Secondly, because patients usually have a choice of treatment programmes, they could reasonably be expected to select one which suited them best (Saunders, 1989). Thirdly, it has been suggested that private treatment programmes may be more accessible to alcoholics with good prognostic features such as employment and favourable psycho-social circumstances to the exclusion of those with personality difficulties (especially psychopathy), depression, or hypochondriasis (Clement and Kahn, 1990). These observations question whether specialized or private treatment centres can empirically

investigate themselves when their set-up is, naturally, more favourable towards their own techniques (Peele, 1990). It remains unclear whether controlled drinking is a more realistic goal than abstinence (see Edwards, 1986 for a synopsis).

The extent of morbidity and mortality as a consequence of amphetamine use in Britain is largely unknown. For example, Stimson and co-workers (1988) found that only 10% of amphetamine injectors were receiving specialist help; in contrast, about 60% of heroin users were receiving treatment. Even in the United States, data on trauma attributable to amphetamine use is not readily available. Nevertheless, an illustrative study of eight American cities (San Diego, San Francisco, Dallas, Phoenix, Los Angeles, Seattle, Denver, and Philadelphia) reported to DAWN showed that between 1985 and 1989, emergency room admissions for amphetamine toxicity almost doubled in the month of March from 1,370 to 2,135; in April for the same years, data from six states (San Diego, San Francisco, Los Angeles, Philadelphia, Oklahoma City, and Phoenix) showed that deaths rose from 65 to 180 (NIDA, 1989 and 1990). Amphetamine use among teenagers and adolescents appears to be on the increase with California having, perhaps, the highest rates. In a representative study of six hundred and fifty nine 16 to 24 year olds admitted to the University of California at Davis Trauma service, 7% tested positive for amphetamine (Helschober and Miller, 1979). Interestingly, while a detailed retrospective case study of 127 patients attending a casualty department with amphetamine toxicity revealed that one-tenth presented with physical complaints suggestive of increased catecholamine activity such as palpitations,

chest pain, seizures, and more rarely, cardiac ischaemia and cerebrovascular accidents, the majority (57%) complained of a deterioration in mental state - agitation, confusion, delusions, hallucinations, and suicidal ideas were common (Derlet et al, 1989). The high rate of perceptual abnormality was not surprising as paranoid psychosis is a well recognized complication of chronic amphetamine abuse (Connell, 1958; Ellinwood, 1967 and 1971).

Like other drug abusers, motivational techniques have been used to encourage cessation of amphetamine misuse (Prochaska and Di Clemente, 1986; Van Bilsen and Von Emst, 1986). After detoxification, relapse prevention strategies which may include the avoidance of *'high risk'* situations, and breaking contact with acquaintances who misuse drugs are encouraged. In human laboratory experiments, extinction to cues have also been tried (Fischman and Foltin, 1991). In Britain, amphetamine misusers are seldom enrolled into residential, rehabilitation programmes. There is no reliable, empirically based information on the outcome of treatment.

In summary, early identification of the alcoholic in the community followed by counselling appears to be a pragmatic approach to treatment. In-patient treatment should, perhaps, be reserved for those with serious medical or psychiatric problems, and a management plan, particularly if treatment is to be long-term, has to be carefully thought out. Psychoanalytical programmes have been ineffective, and none of the other psychological regimes stands out from the rest. The alcoholic should be guided to chose one which suits best.

Prolonged abstinence following treatment is uncommon and may be an unrealistic goal. In Britain, it is obvious that there is a pressing need for a nation-wide database to monitor morbidity and mortality following amphetamine use and misuse, and to evaluate treatment outcome. Though extrapolation from the United States situation must be cautious, there is concern that the use of amphetamine and amphetamine-like substances may be growing in urban areas with increasingly younger children becoming involved, and that this may be an important contributory factor to the spread of the HIV virus.

2.3. Pharmacological treatments for alcohol abuse

In the last two decades, pharmacological treatments for drug addiction have become increasingly popular. In general, the main focus of medical attention has been with ameliorating the withdrawal symptoms of drugs of abuse including alcohol. It was held, perhaps naively, that uncovering how neurones adapt to these drugs would explain the biological determinants of the addictive process. At present, the pharmacological management of alcohol withdrawal remains central to drug treatment approaches.

The search for specific antagonists to the acute effects of alcohol has proved fruitless. The anecdotal *'sobering-up'* properties of caffeine cannot be explained by its specific psychotropic actions (Nuotto et al, 1982). Additionally, naloxone's ability to alleviate alcohol-induced coma can, simply, be attributed to amelioration of the exacerbating effects of endogenous opioids

(Dole et al., 1982). The benzodiazepine antagonist RO 15-4513 (ethyl-8-azido-5,6-dihydro-5-methyl-6-oxo-4Himidazo[1,5a][1,4]benzodiazepine-3-carboxylate) reduces some of the behavioural effects of ethanol, and inhibits ethanol-stimulated chloride ion fluxes. It, however, does not reverse the lethal effects of alcohol in animals, and its epileptogenic properties mitigates against safe therapeutic use in humans. RO 15-4513 is not a specific antagonist to the acute effects of alcohol; it also antagonizes the action of other sedatives including barbiturates (for a brief review, see Lister and Nutt, 1987).

Traditionally, the avoidance of aversive stimuli or withdrawal symptoms was considered to be critical to maintaining drug-seeking behaviour. Thus, it is not surprising that the use of pharmacological agents to condition aversive responses to drinking was a major treatment goal. Emetine-induced nausea was used almost half a century ago to prevent drinking, and more recently, there was a brief flirtation with apomorphine. Lately, disulfiram and similar chemical agents have been used to condition aversive responses (Kreek, 1987). The therapeutic efficacy of disulfiram is doubtful. It has mainly been shown to be of benefit among alcohol abusers who attend regularly, have a high expectation of success, and who are well supported by their spouse and family (Kitson, 1977); that is, the sort of abuser most likely to refrain from drinking without them. Hence, producing aversion may, in itself, be insufficient to prevent the reinstatement of drinking. It has, therefore, been argued that giving disulfiram is nothing more than administering a *'psychological pill'* - each time it is taken,

the alcohol abuser is reminded of the therapeutic bond and treatment contract with his or her therapist.

Historically, another important approach has been to break the vicious cycle of alcohol-induced dysthymia leading to increased drinking by prescribing lithium - a drug whose antidepressant properties have been attributed to stabilization of 5-Hydroxytryptamine (5-HT) neurotransmission by simultaneously increasing 5-HT brain levels and decreasing the number of 5-HT receptors (Bunney and Gerald-Bunney, 1987). Depressed drinkers benefited most with improved mood and a reduction in alcohol consumption (Merry, et al., 1976; Rounsaville et al., 1982), and a decrease in the urge to drink (Judd and Huey, 1984). Lithium therapy was abandoned because the erratic fluid intake of alcohol abusers often precipitated toxicity. Interestingly, preliminary reports suggest that other drugs with actions on 5-HT neurotransmission such as fluoxetine may also reduce alcohol consumption (Naranjo et al., 1990).

2.4. Pharmacological approaches to amphetamine misuse

The principal pharmacological activity of amphetamine at clinically relevant doses is to produce a rise in the availability of dopamine at the synaptic cleft by potentiating release; additionally, there is some enhancement of neurotransmission at both noradrenergic and 5-HT receptors, and it is a weak inhibitor of amine re-uptake (Carlsson, 1970; Heikkila et al, 1975). The psychological effects of amphetamine including its ability to act as a reinforcer,

and thereby maintain drug-seeking behaviour (see below for details), appear to be mediated by dopamine. While specific antagonists at both the D_1 and D_2 receptor subtypes of dopamine attenuate amphetamine-induced reinforcement in animals (Yokel and Wise, 1976), and D_2 receptor blockers inhibit the mood-inducing effects of amphetamine in humans (Jacobs and Silverstone, 1986), the risk of triggering extrapyramidal dysfunction, especially tardive dyskinesia, precludes their long-term use to treat amphetamine abuse. Emergency or short-term use of a D_2 receptor antagonist is sometimes necessary to reduce the severity and duration of amphetamine-induced psychotic symptoms.

3. Synopsis of new approaches to drug addiction: behavioural correlates - the importance of reinforcement and the discriminative stimulus effect

In recent years, conditioning and learning processes have been recognized to be critical in directing, sustaining, and controlling, drug seeking behaviour. For a drug to have abuse potential it must have two essential properties which allow it to act as a psychostimulant (Wise and Bozarth, 1987).

Firstly, it should have the ability to act as a positive reinforcer, whereby the likelihood and frequency of drug-seeking behaviour is increased when the drug is presented. In this respect, most abused drugs have psychostimulant properties.

Secondly, it must be able to produce an internal stimulus (discriminative cue) that can be readily distinguished by the animal. In this way, characteristic subjective effects of classes of drug can be readily recognized, and differentiated from other drugs and the non drug condition.

In addition, some abused drugs (e.g. alcohol) may possess aversive properties but this of less importance in maintaining drug-seeking behaviour. That is, in the naive individual, mild unpleasant effects, such as dysphoria or anxiety, may occur when the drug is taken. Nevertheless, with further exposure, the appreciation of its positive subjective effects strengthen considerably and little or no aversive symptoms are experienced provided the individual does not become intoxicated. Alcohol dependent individuals usually experience few positive subjective effects on drinking but develop severe and sometimes prolonged withdrawal symptoms such as anxiety and depression on abstaining. Thus, avoidance of withdrawal may play a part in shaping alcohol-seeking behaviour in alcoholics.

In summary, four principles govern the new approach to drug addiction. They are: (i) drug-seeking behaviour is common to all drug addictions; (ii) all drugs of abuse can act as psychostimulants; (iii) the reinforcing and discriminative stimulus effects of drugs of abuse are critical to directing, controlling, and maintaining drug-seeking behaviour; and (iv) the avoidance of

withdrawal symptoms or aversive stimuli may influence drug-seeking behaviour in some classes of drug such as alcohol (Stolerman, 1992).

4. SCIENTIFIC BASIS OF DRUG-SEEKING BEHAVIOUR

4.1. Background and concepts of behavioural measures

There has been much confusion in the literature because the terms *reward* and *reinforcement* have been used interchangeably. Reinforcers produce response-contingent stimulation; that is, they enhance or sustain the probability that the response will be repeated (Skinner, 1932). This must be differentiated from *priming effects* where stimulation increases the incentive or drive to carry out the response in a non response-contingent manner. In this respect, and in the present thesis, *reward* refers to the integral activity of both *priming* and *reinforcement* processes.

In animal experiments, electrical self-stimulation of certain brain *reward* sites produce a rise in rates of responding over a *dynamic range* which is in proportion to the strength of the stimulus. Eventually, a plateau is reached, whereby greater stimulation leads to no change in response above or below a certain level. This plateau, or *asymptotic range*, is not related to the reinforcing value of the stimulus but gives the limit of responding for that specific brain region (Miliaressis and Malette, 1987; Waraczynski et al., 1987). Limits of responding vary between brain regions (Prado-Alcala and Wise, 1984). The *asymptotic range* is dependent on motor function and can be impaired by simultaneous stimulation at different sites (Miliaressis and Rompre, 1987); in

contrast, the *dynamic range* is a reflection of the reinforcing value of a particular stimulus or drug of abuse. Early experiments which used *rate-dependent* measures (or *reward thresholds*) have been heavily criticized because it was difficult to determine if, and by how much, variations in response rate for a given putative reinforcer could be attributed to changes in either the *asymptotic* or *dynamic range,* or both (Valenstein, 1964). For instance, *reward thresholds* could be defined in three ways: a cut-off point above which stimulation produces responses more than 50% of the time, half the *asymptotic* level, or the minimum stimulation needed to maintain rates of responding above the baseline. The important problem was that while *asymptotic* and *dynamic* rates could only be segregated at the *asymptotic* point (i.e. at a point of stasis), variations in the *dynamic range* always influenced the *asymptotic* level. Thus, differentiation between the effect of the reinforcer on response, and the limit of responding to it was not possible, and discrimination between responses which occurred in the *asymptotic or dynamic ranges* was unclear (Valenstein, 1964). Nowadays, the *curve shift* paradigm is the psychometric measurement of choice. For instance, the plot of response rate Vs the log of the stimulation intensity is usually sigmoidal. While a biologically significant shift to the right (i.e. to higher stimulation intensities) indicates a decrease in the reinforcing effect of the stimulus or drug of abuse, a shift to the left implies the converse. Notably, experimental *noise* can sometimes produce small but biologically insignificant lateral shifts. Increased performance capacity leads to upward shifts, and *vice versa.* In essence, these curves are analogous to dose-response relationships in pharmacology: here, the stimulus intensity is

the drug dose. In summary, the *curve shift paradigm* has been used to identify and chart the relative contribution of a range of brain sites to the reinforcement process, and to evaluate the differential strengths of a variety of reinforcers at such sites (Diagram 1).

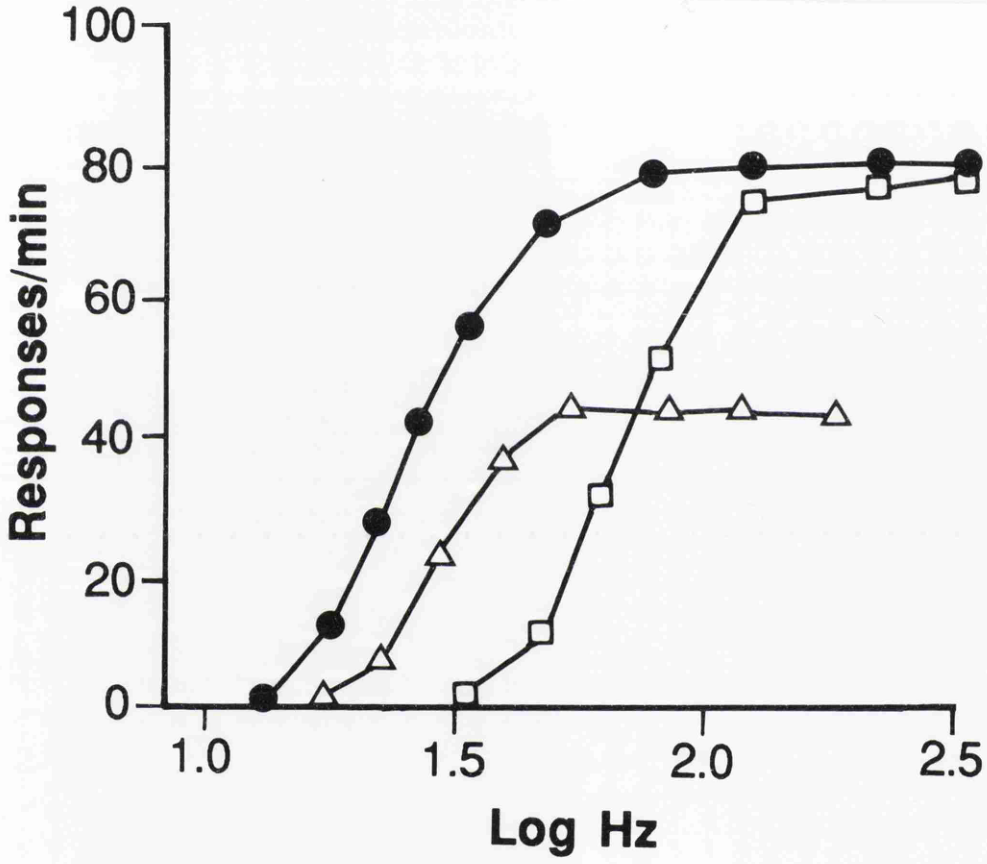

Diagram 1: *is a rate-frequency 'curve-shift', whereby response is plotted against the log of the number of pulses in a burst of 'reinforcing' stimulation. Filled circles depict the baseline curve. A decrease in reinforcing stimulation, for example by the administration of a moderate dose (0.25 mg/kg) of the D_2 receptor antagonist, pimozide, shifts the curve to the right (open squares). At higher doses, 0.5 mg/kg, motor impairment of responding shifts the curve downwards (open triangles).*
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4.2. Conditioned Place Preference (CPP)

The CPP paradigm is a well known rate independent method for assessing the reinforcing impact of electrical stimulation, drugs of abuse, food, and taste preference. Unlike self-stimulation which is dependent on eliciting stimulus contingent responding, CPP relies on the ability of the reinforcer to cause approach behaviour.

In CPP a neutral stimulus is *paired* with the reinforcer such that the stimulus itself becomes capable of serving as a reinforcer (*secondary conditioning* {Diagram 2}). While there is considerable evidence that CPP is mediated by dopaminergic pathways from the VTA to the nucleus accumbens, other neurochemicals and non-dopaminergic mechanisms, as in intracranial self-stimulation (ICSS) described below, may play a modulatory role. Dopamine receptor antagonists injected into the nucleus accumbens decrease CPP induced by drugs of abuse such as amphetamine (Mackey and Van der Kooy, 1985; Mithani et al., 1986); additionally, lesions in the tegmentum (Bechara and Van der Kooy, 1986) and nucleus accumbens (Spryaki et al., 1982a) can reduce CPP, while destruction of central noradrenergic fibres does not (Spryaki et al., 1982a and b).

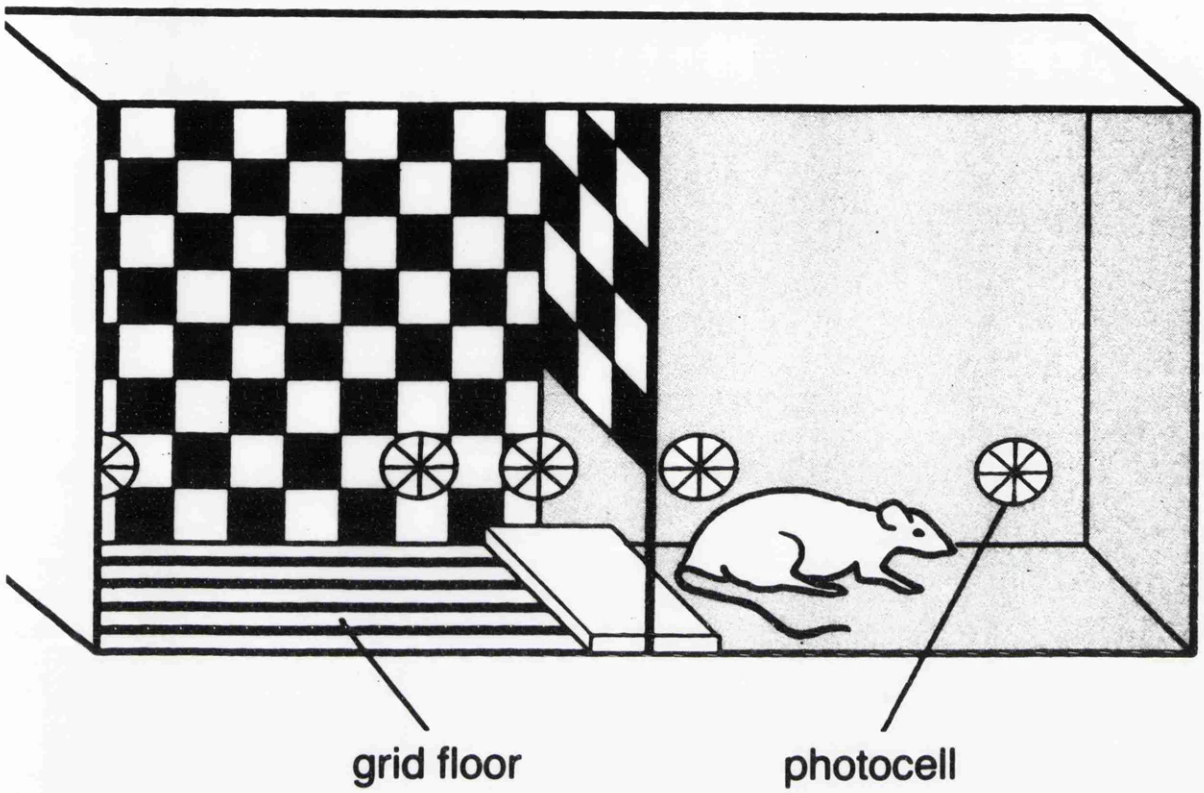

Diagram 2: *A simple CPP box has two different enclosed compartments separated by a guillotine. While one compartment is predominantly featureless, the other has a grid floor and chequered walls. The animal receives the reinforcer (e.g. a drug of abuse) in one compartment, and vehicle in the other. After 'pairing' the animal in this way, the guillotine is lifted. The animal can now roam freely between the two compartments. The amount of time the animal spends in each compartment can be measured by light beams or photoelectric cells. Treating the animal with a dopamine antagonist reduces the time it spends in the chamber in which it was 'paired' with the drug of abuse.*

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4.3. Mapping brain reinforcement pathways and Intracranial self-stimulation (ICSS)

Reinforcement is critical to the direction, control, and maintenance of drug-seeking behaviour. Neuroanatomical pathways involved in reinforcement have been mapped by electrical stimulation of specific brain regions, and the action of drugs of abuse at certain receptor sites.

Using movable electrodes to demarcate the boundaries of the reinforcement circuit, Schmitt et al (1974) discovered that aminergic fibres in the mesencephalon - a combination of serotonergic, dopaminergic, and noradrenergic fibres - were associated with reward. Stimulation of other regions, notably the periacqueductal grey, was aversive. The experiments of Corbett and Wise (1980) provided evidence that dopaminergic fibres were essential to reinforcement as low thresholds for responding were found in the ventral tegmentum area (VTA); further, high threshold reinforcement sites were located superior to the VTA. This suggests that the mesencephalic reinforcement circuits are extensions of, or in close association with, the tegmental network (Rompre and Miliaressis, 1985). While stimulation of the locus coeruleus produced no reinforcement suggesting that noradrenergic pathways were not primarily involved with reinforcement, noradrenergic pathways were found to be important for *priming* the animal for locomotion, thus enabling the expression of the response.

ICSS is a special type of operant conditioning, whereby the animal learns to self-administer electrical stimulation as a surrogate to a natural reinforcer such as food or water. ICSS has been demonstrated throughout the length of the medial forebrain bundle (for a review see Phillips and Fibiger, 1989). The findings of ICSS experiments suggest that dopaminergic fibres, particularly in the ventral tegmental, may mediate reinforcement process, and that the principal sites extend from the anterior region of the nucleus accumbens to the level of the mesencephalic nucleus of the trigeminal nerve (Rompre and Boye, 1986). Other strong candidates for reinforcement sites include the medial prefrontal cortex and cingulum (Silva et al., 1982). The lateral hypothalamus (Edmonds and Gallistel, 1974; Miliaressis et al., 1986), septal nuclei (Waraczynski, 1988), amygdala (Gallistel et al., 1985), habenula (Nakajima, 1984), and cerebellum (Watson, 1978; Corbett and Wise, 1982) have also been suggested as possible sites. In addition, there is evidence that other neurochemicals modulate the mesocorticolimbic reinforcement circuit. These include: neuropeptides (Crow and Deakin, 1985); cholecystokinin {CCK} (Hsiao and Deupree, 1983; Ettenberg and Koob, 1984); gamma-aminobutyric acid {GABA} (Kent and Fedinets, 1976; Zarevics and Setler, 1981); acetylcholine {Ach} and adrenocorticotrophic hormone {ACTH} (Izquidero, 1984). Serotonin systems might also affect the expression of reinforcement-seeking behaviour. Para-chlorophenylalanine (PCPA), which inhibits 5-HT synthesis, decreases ICSS in the dorsal (Van der Kooy et al., 1978) and median (Miliaressis, 1977) raphe, hippocampus (Van der Kooy, 1977) and caudate nucleus (Phillips et al., 1976). Notably, the attenuation of

ICSS in the medial forebrain bundle (MFB) is, temporally, out of phase with 5-HT depletion suggesting other inter-related neural mechanisms may be operating (Stark et al., 1970). Paradoxically, some studies have reported an increase in ICSS following PCPA or neurotoxic destruction of the 5-HT system (Gibson et al., 1970; Porschel et al., 1973). In addition, self-stimulation of 5-HT fibres which arise from the dorsal raphe is attenuated by serotonin blockade (Nakajima, 1984). Thus, it is conceivable that interruption of 5-HT activity simply makes the animal work harder to maintain the same level of reinforcement. Taken together, this suggests that the 5-HT system plays a modulatory rather than a direct role in reinforcement . It is also of note that while drug injection studies have suggested acetylcholine may also modulate reinforcement, confirmation of this from neuroanatomical studies has not been forthcoming (Yoemans et al, 1985). The ways by which non-dopaminergic neuronal circuits might influence the putative mesolimbic dopamine reward pathway are described in more detail elsewhere (section 5).

4.4. Alcohol-induced reinforcement: further evidence of dopaminergic involvement

Alcohol, like other drugs of abuse, can function as a reinforcer (Meish, 1984). It has been suggested that the mesocorticolimbic dopamine system plays an important role in the mediation of alcohol-induced reinforcement. For instance, in non-deprived (of food and water) rats, dopamine receptor antagonists decrease lever-pressing for alcohol (Pfeffer and Samson, 1985;

Samson et al., 1988b), and diminish home-cage ethanol drinking. Non-deprived Wistar rats that have been trained using a two-lever, free-choice, self-administration task, whereby the animal is allowed to make a choice whether to press one lever to receive nutrients (food and water) or the other to get the surrogate reinforcer, show decreases in stimulation-induced responding and in the amount of alcohol consumed when the dose of alcohol (within the range of 5-10% alcohol content by volume) is reduced; an increase in dose produces the opposite effect on both responding and consumption (Samson et al., 1988b).

The classical experiments of Overton (1901) - see Lipnick, 1986 for a review - suggested that the acute actions of alcohol was dependent on its ability to *fluidize* neuronal membranes, and that tolerance to chronic alcohol consumption could be explained by increased membrane *rigidity*. While this view was still held by some investigators until the early 1980's, it become clear that these structural membrane distortions were, in themselves, insufficient to explain changes in cell function (Dietrich et al., 1989; Buck et al., 1991).

Neurochemical studies have also been used to segregate the differential role of catecholamines in reward. Intravenous ethanol has been shown to increase cell firing in the VTA of awake (Gessa et al., 1985) but not anaesthetized rats (Mereu et al., 1984). Gessa and co-workers (1985) suggested this action was indirect: ethanol suppressed $GABA_A$ neurones that were tonically inhibiting the firing of dopaminergic neurones; interestingly, an

electrophysiological study of oocytes expressing cloned GABA receptor subunits has suggested that alcohol-induced GABAergic activity essentially takes place at a specific γ unit, γ_{2L} (Wafford et al., 1991). In rats, the injection of dopamine receptor antagonists into the nucleus accumbens leads to a reduction in oral ethanol self-administration (Rassnick et al., 1992). In addition, administration of a small dose of ethanol increases locomotor activity (Waller et al., 1986), raises extracellular levels of dopamine in the nucleus accumbens (Imperato and Di Chiara, 1986), and potentiates cation conductance at 5-HT_3 receptors (Peters et al., 1992). In alcohol-preferring P rats that were orally self-administering small doses of alcohol, this rise in extracellular dopamine was greater than that seen in non alcohol selective strains (Weiss et al., 1992). It is worth stressing that the reinforcing effects of alcohol have been demonstrated using small doses (3-10% alcohol content by volume). At higher doses, other receptor mechanisms, notably $GABA_A$, may have an enhanced neuromodulatory role. At intoxicating doses, profound motor impairment makes the meaningful interpretation of responding impossible, and there are considerable changes at a wide variety of receptor sites. In particular, there is further enhancement at $GABA_A$, and decreased activity at NMDA and Kainate receptors and at Voltage-sensitive calcium channels. The finding that rats selectively bred to prefer ethanol may, comparatively, release more extracellular dopamine on its administration may have implications for the study of the genetic basis of alcoholism in humans. In this respect it is of note that the A1 allele of the dopamine D_2 receptor gene may be over-represented in alcoholics (Blum et al, 1990), and that carriers of the A1 allele have fewer

D_2 receptors than those with the A2 allele (Noble and Blum, 1991); in gainsay, these findings have not been replicated by others (Bolos et al, 1990; Parsian et al, 1991), and should be regarded as preliminary.

The central role of dopamine in reinforcement has also been supported by evidence from functional studies of calcium channel inhibitors (CCI). In animals, CCI decrease alcohol preference and intake, presumably by antagonizing dopamine-mediated reinforcement and the discriminant stimulus effect (Engel, 1988). Nevertheless, reduced ethanol consumption by CCI could also be due to taste aversion - a factor which may explain the prolonged inhibitory action of Goe 5438 (Pucilowski et al., 1992). CCI may have a therapeutic role in the management of alcohol dependence and withdrawal. Chronic alcohol exposure leads to up-regulation of voltage-sensitive Ca^{2+} channels and an increase in intracellular calcium; concomitantly, GABA activity is reduced and NMDA sites up-regulated. Thus, chronic alcohol produces chemical changes that antagonize the acute effects of alcohol, leading to a state of hyperexcitability when it is withdrawn. In animals, CCI attenuate tremor, hyperexcitability, and seizures due to alcohol withdrawal (Dolin et al., 1987; Bone et al., 1989) by, presumably, redressing some of this chemical imbalance. In animals, CCI also inhibits conditioned place preference to other drugs of abuse such as cocaine and *d*-amphetamine (Fatta et al., 1991; Pucilowski 1992), and has been shown to reduce the intake and positive subjective effects of cocaine (Jaffe 1989) in man, presumably by decreasing its reinforcing properties.

4.5. Neurochemical effects of alcohol on noradrenergic pathways

The effect of ethanol on the locus coeruleus is principally, but not always, depressant. Pohorecky and Brick (1977) showed that i.p ethanol at a dose of 2g/kg in paralysed rat preparation mostly produced inhibition of cell firing; 22% of the cells were activated and 16% were unchanged. In addition, when ethanol was directly applied to the neurones attenuation of cell firing was almost universal. Ethanol-induced inhibition of cell firing was also seen in animals anaesthetized with chloral hydrate (Strahlendorf and Strahlendorf, 1983). From *in vitro* studies, cell firing is attenuated in brain slices perfused with ethanol at concentrations between 1-60 mM (Shefner and Tabakoff, 1984). The main effect of ethanol on noradrenergic pathways is, therefore, depressant. The interaction between noradrenergic and dopaminergic mechanisms in the expression of reinforcement is discussed below (section 5).

4.6. Amphetamine-induced reinforcement: additional evidence of dopaminergic involvement

Rats can be trained to lever press to self-administer amphetamine intravenously (Pickens and Harris, 1968; Wise et al, 1976). It is also well recognized that dopamine receptor agonists such as apomorphine and piribedil administered on their own (Davis and Smith, 1977; Yokel and Wise, 1978), and in the presence of *d*-amphetamine (Wolverton et al, 1984), can maintain

responding in rats. While the selective D_1 agonist, SKF 38393, did not sustain responding, pre-treatment with either the D_2 antagonists, haloperidol (Davis and Smith, 1975) or pimozide reduces responding after an initial increase which might reflect a *'rebound'* phenomenon (i.e. an *"extinction burst"*) to try and overcome the blockade or a reduction in the antagonist's rate-decreasing properties, or both. Yokel and Wise (1985) found that substituting pimozide pre-treatment with saline produced similar extinction on responding for amphetamine. Importantly, the direct adrenergic agonist, methoxamine (Riser and Jones, 1976), and the adrenergic re-uptake blocker, nisoxetine (Woolverton, 1987), do not maintain lever pressing; further, adrenergic antagonists such as phenoxybenzamine and propanolol have no effect on amphetamine-induced responding. Taken together, the D_2 receptor appears to be more directly involved in amphetamine-induced reinforcement than D_1; in contrast, the D_1 receptor may mediate drug discrimination cues to amphetamine (for a review see Neilsen and Anderson, 1992). Though adrenergic pathways do not appear to mediate amphetamine reinforcement, enhanced noradrenergic neurotransmission appears to be important in *priming* and activation of *'burst'* firing of dopaminergic neurones - assumedly to stimulate and maintain cognitive processes during stress or arousal (D'Angio et al, 1988) and preserve selective attention (Oades, 1985).

Convincing evidence that central dopaminergic pathways are critical to amphetamine reinforcement has been demonstrated by microinjection, *CPP*, and lesion studies. Direct injection of amphetamine into the nucleus accumbens

produces hyperactivity contingent on the release of endogenous dopamine (Pijnenburg et al, 1976; Costall, 1980; Kuczenski et al, 1991). *D*-amphetamine reliably produces *CPP* (Mithani et al, 1986; Leone and Di Chiara, 1987; White et al, 1987), and selective D_2 receptor antagonists such as haloperidol (Mithani et al, 1986) and α-flupenthixol (Mackey and van der Kooy, 1985) abolish it. Further, 6-hydroxydopamine (6-OHDA) lesions of the nucleus accumbens attenuates amphetamine self-administration (Lyness et al, 1979; Roberts et al, 1980; Roberts and Koob 1982). Interestingly, *d*-amphetamine also enhances control over behaviour expressed by conditioned reinforcers - a role attributed to dopamine-mediated incentive learning (Robbins and Koob, 1978; Beninger et al, 1981; Robbins et al, 1983). In a classic experiment, Taylor and Robbins (1984) showed that thirsty animals could be trained to reliably associate a dipper noise (the conditioned stimulus {CS}) with the delivery of water (the unconditioned stimulus{US}). Of the two levers in the animals' cage, one when pressed (the conditioned response {CR} lever) produced the CS and the other (the non-conditioned response {NCR} lever) on pressing elicited nothing. The trained animals responded more on the CR than the NCR lever. Following intra-accumbens injection of *d*-amphetamine, responding on the CR lever was increased while no enhancement of lever pressing was seen on the NCR. Behavioural specificity was confirmed by demonstrating that increased CR responding on the CR lever did not occur without association of the CS with the US. Neurochemical specificity was demonstrated by showing that intra-caudate injection of *d*-amphetamine produced increased responding on the CR lever Biochemical specificity was illustrated by showing that intra-accumbens

6-OHDA lesions, but not noradrenergic lesions (Cador et al, 1991) stopped the rise in responding on the CR following *d*-amphetamine (Taylor and Robbins, 1986); however, following saline infusion (i.e. without amphetamine) behavioural control exerted by the CR is not altered. Thus, dopamine neurotransmission in the ventral straitum both facilitates and augments amphetamine-induced amplification of contingent behaviours, and other (i.e. non-dopaminergic), perhaps even limbic mechanisms, may be involved in the mediation of responding to naturally conditioned stimuli under non-contingent (i.e. everyday) circumstances.

4.7. Amphetamine and cognitive performance: from humans to important lesion studies in animals

It is well established that physiological doses of amphetamine in humans (between 2.5 mg to 30 mg orally) increase speed of response, vigilance and attention, motor performance, and learning on tests of memory (Weiss and Laties, 1962; Gunne, 1977; Spiegel, 1979; Harvey, 1987). In a recent illustrative study, six healthy male volunteers (aged between 21 and 38 years) were subdivided into two groups of three. Each subject received active and placebo *d*-amphetamine (0 or 10 mg/70 kg) twice daily during two trials each held on three consecutive days. Placebo and drug were administered alternately, and the order of exposure was counter-balanced between the two groups. In amongst other subjective ratings of mood, general well-being, and social behaviour, subjects performed computerized tests of vigilance, the

vigilance task, and learning, the digit-symbol substitution task (DSST). Subjects showed a significant increase in vigilance and DSST accuracy and in performance with no increase in false alarm rates on responding (Kelly et al., 1991).

It is of interest that lesions studies of the nucleus accumbens have been used in an attempt to tease out the differential contribution of noradrenergic and dopaminergic pathways to attention and arousal. Cole and Robbins (1987) subjected rats with 6-OHDA lesions of the dorsal noradrenergic bundle (DNAB), which reduced cortical noradrenaline to within 5% of control levels, to systemic and nucleus accumbens infusion of amphetamine and administered an adaptation of Leonard's five choice reaction time test developed for humans. These lesioned rats showed no diminution in performance. Though sham-operated rats (controls) responded more impulsively following amphetamine, there was no impairment of discriminative accuracy; in contrast, the lesioned rats showed impairment of discriminative accuracy - an effect which was attenuated by the D_2 receptor antagonist, α-flupenthixol. Taken together, these results suggest that attention depends on dopaminergic activity in the nucleus accumbens, and as was shown in a later experiment (Cole and Robbins, 1992), forebrain noradrenergic pathways may be involved in controlled or 'effortful' processing.

The present thesis contains the first investigation of 5-HT_3 receptor blockers, which oppose dopaminergic activity in the nucleus accumbens, on

amphetamine-induced changes in reaction time and attention; this investigation is made more intriguing by the finding that 5-HT_3 receptor antagonists may have cognitive enhancing properties in animals (Costall et al., 1990), possibly by facilitation of acetylcholine neurotransmission (Barnes et al 1989a and b).

4.8. Amphetamine: effects on caloric intake and macronutrient selection

From animal studies, there is direct evidence that feeding behaviour is largely mediated via complex interactions between dopamine, noradrenaline, and 5-HT (which is discussed later). For instance, adrenaline and noradrenaline reduce food intake in rats fed high carbohydrate diets (Russek et al., 1976), dopamine receptor agonists such as L-dopa (Sanghvi et al, 1975) and piribidel (Carruba et al, 1980) diminish feeding in food deprived animals, and the anorexic effects of the β-adrenergic agonist, salbutamol, are antagonized by the β-adrenergic receptor antagonist, propanolol.

The anorectic properties of amphetamine have been studied for over forty years. *D*-amphetamine enhances the release of both noradrenaline and dopamine (Carlsson, 1970). In addition, brain stimulation, lesioning, and behavioural studies in animals suggest that it is this increased catecholamine availability that is responsible for amphetamine's ability to suppress food intake. Anorexia induced by amphetamine (at a dose of 1.25 mg/Kg) and mazindol (7.5 mg/Kg) is blocked by lesions of the ventral noradrenergic bundle

(Samanin et al, 1977), and disruption of catecholamine synthesis by alpha-methyl-para-tyrosine reduces food intake (Baez, 1974). Notably, amphetamine-induced anorexia is only partially attenuated by dopamine receptor blockade with α-flupenthixol at relatively high (Garattini and Samanin, 1976) but not at low dosages (Burridge and Blundell, 1979). Hence, it has been suggested that noradrenergic rather than dopamine stimulation may be more critical to maintaining anorexia following amphetamine (Samanin et al., 1978; Samanin and Garattini, 1982).

Microinjection of putative anorectic agents into specific brain regions have been used to locate neuroanatomical substrates for the control of feeding. Two brain regions in the lateral hypothalamus with differential activity on food intake have been isolated. In animals, injection of α-adrenergic agents, into the paraventricular area stimulates feeding (Leibowitz, 1978); while infusion of serotonin or fenfluramine into the same brain area attenuates feeding (Leibowitz and Papadakos, 1978). In contrast, infusion of dopamine and β-adrenergic agonists into the perifornical zone inhibits food intake (Leibowitz and Brown, 1980). Further, injection of amphetamine into the perifornical area reduces food intake (Leibowitz and Rossakis, 1979). In future, elaborations on this model will have to take into account our growing knowledge on the role of endorphins, peptides, and other neuromodulators such as GABA agonists (Blundell, 1981) and CCK (Gibbs et al., 1973; Kissileff et al., 1981) on feeding (for a discussion see Hoebel, 1984).

Food deprivation studies have been widely used to investigate feeding behaviour and food intake in animals. In these experiments, the animal is allowed food for 1-2 hours, having been denied it for 16 to 48 hours. The amount of food consumed when access to food is granted represents the anorectic potential of the drug with which it had been pre-treated. There have, however, been five main reservations about the use of the food deprivation and traditional paradigms to test the anorectic potential of drugs. Firstly, severe fasting may itself be physiologically traumatic (Blundell and Latham, 1982) - thus, inferences drawn from this approach may be unlike feeding behaviour in non-deprived animals. Secondly, observations of feeding behaviour are made in deprived animals, and their neurochemistry is studied when they are satiated (Glick et al, 1973). Thirdly, solely measuring caloric intake is misleading as it provides no clue as to what component of the feeding process is impaired. Fourthly, consuming bland and novel laboratory foodstuffs within pre-determined time limits does not allow for the expression of certain eating behaviours in animals such as foraging and hoarding - thus, it has been suggested that examination of drug effects using 'free-feeding' paradigms and sequencing while continuously monitoring food selection may be more appropriate (Blundell, 1982). Fifthly, food deprivation is an unnaturalistic model of animal and even normal human consumatory behaviour whereby feeding periods are interspersed with non-feeding intervals (i.e. satiety produced by the food itself is also anorectic), and the opportunity of selection from a variety of foodstuffs of different macronutrient compositions, taste and texture is often afforded.

It is now widely accepted that animals are able to discriminate and select their intake of macronutrients. For example, rats allowed access to fat, protein, carbohydrate, minerals, and water are able to maintain a balanced diet (Overmann, 1976). Voluntary self-selection is thought to be under the control of neurotransmitters. Amphetamines reduce carbohydrate and protein (McArthur and Blundell, 1983) and fat intake (Marks-Kaufman and Kanek, 1980) in non food deprived animals; paradoxically, both a small dose of amphetamine, 0.125 mg/Kg (Blundell and Latham, 1978) and lateral hypothalamectomy (Stricker and Zigmond, 1976) appear to increase food consumption in non-deprived rats. Amphetamines also have notable effects on feeding behaviour. When given to rats they eat quicker; additionally, pre-treatment with the clonidine, an α-adrenergic agonist, will increase carbohydrate selection (Mauron et al, 1980). It appears that the main contribution of dopaminergic mechanisms to feeding might be to impair the regulation of sweet foods. For instance, selective D_2 receptor and D_1antagonists suppress sucrose intake in sham feeding in rats (Shneider et al., 1986).

In humans, physiological doses of amphetamine (2.5 mg to 30 mg orally) has been shown to reduce appetite (with a peak at about 2 hours post drug) and, less strongly, caloric intake; additionally, initiation of eating might also be impaired (Silverstone and Stunkard, 1968). As in animals, the differential effects of noradrenaline and dopamine on feeding have been

studied. In a preliminary study, thymoxamine (a noradrenergic blocking agent) attenuated the anorectic properties of subjects pre-treated with *d*-amphetamine but spared its euphoriant effects; it was later shown that this mood enhancement could be attenuated by the selective D_2 receptor antagonist, pimozide (Silverstone and Kyriakides, 1982). However, the effects of thymoxamine on amphetamine-induced anorexia was not replicated (Goodall et al., 1987). Importantly, in a recent study which compared the effects of *d*- and *l*-fenfluramine and *d*-amphetamine on energy and macronutrient intake, it was shown that d-amphetamine alone produced a small but statistically insignificant decrease in sweet food intake; this reached statistical significance when *d*-fenfluramine was given with *d*-amphetamine. *D*-amphetamine (15 mg orally) also reduced feeding time and caloric intake (Goodall et al., 1992). It is worth noting that the overall percentage decrease following *d*-amphetamine alone was considerable (15%), and may be biologically significant, and that a study with a larger number of subjects and less inter-subject variability might have been able to draw more definitive conclusions. Further studies using *'temporal tracking'* of eating behaviour are needed, whereby hunger and desire or motivation to eat are examined at pre-determined stages of feeding. Dissociation between these parameters might indicate hunger *per se* may not control intake once feeding has been initiated.

In sum, in animals and humans, amphetamines have observable effects on the reduction of caloric intake and feeding time, and probably diminish sweet food intake and preference.

5. The mesolimbic dopamine system and other important pathways in the reinforcement process: a theoretical framework for explaining the translation of motivation into behaviour

It has been hypothesised that the 'limbic-motor interface' (Mogenson et al, 1980; Heimer et al, 1982) is critical to the organization, direction, and maintenance of a co-ordinated response to changes in environmental conditions. Simply, biologically important changes in environmental conditions are carried as afferent stimulation, chiefly from the amygdala and hippocampus, to the ventral tegmental area (VTA). In the VTA, these afferent impulses are quantified and assessed (i.e. 'gated') by the nucleus accumbens (i.e. the 'interface') which sends the efferent response to the motor system.

The neural connections between afferents to the A10 dopamine containing cells of the VTA and efferents from the nucleus accumbens is highly complex and imperfectly understood. The simplified schematic diagram of a rat brain (saggital section) below summarizes the essential elements and the principal neurotransmitter systems involved (Diagram. 3).

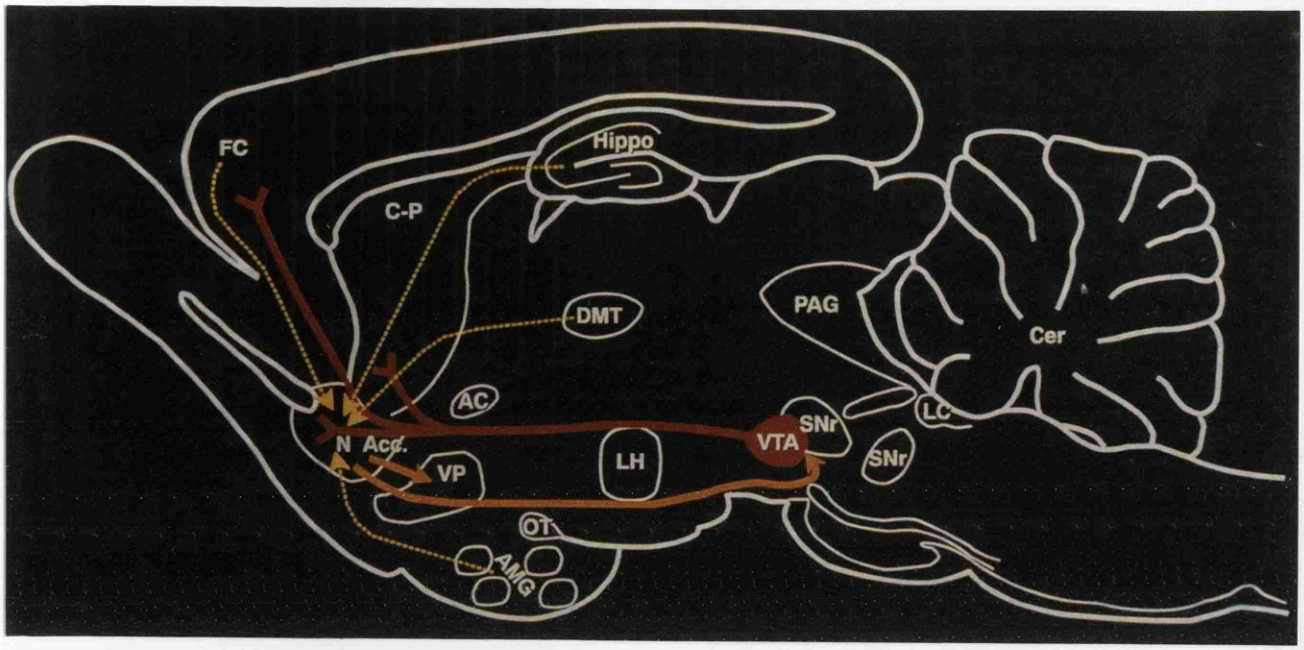


Diagram. 3. *Limbic afferents (yellow) to the nucleus accumbens are of two functionally important types: (a) fibres from the central nucleus of the amygdala (constituting the amygdala - ventral straitum - ventral pallidum axis) and (b) those from the hippocampus (Hippo). In addition, there are glutaminergic afferents from the dorsomedial thalamus (DMT) and the frontal cortex. Serotonergic afferents from the median and dorsal raphe bundles are not shown. Further, there is noradrenergic afferent innervation of the nucleus accumbens by the locus coeruleus (LC). It has been suggested that the serotonergic and noradrenergic innervation provide non specific arousal of the A10 dopamine containing cells of the VTA. However, regulation of A10 neurones by 5-HT shows evidence of functional heterogeneity. For instance, while both dorsal and median raphe bundles attenuate firing of A10 cells, median raphe fibres also stimulate dopamine containing cells from the VTA to the prefrontal cortex (Hervé et al, 1981); hence, this inhibition of dopamine may be mediated by 5-HT_2 fibres (Ugedo et al, 1989). Interestingly, injection of 5-HT into the VTA increases dopamine release (Guan and McBride, 1989). 5-HT_3 receptor antagonists inhibit this release (Costall et al., 1987) and attenuate firing of both A9 and A10 dopamine containing cells (Sorensen et al., 1989). In addition to the usual 'pacemaker' or background activity, A10 dopamine neurones are capable of 'burst firing' in specific VTA areas to direct and co-ordinate the response to the stimulus and activate the cognitive processes necessary to deal with it (D'Angio et al., 1988). The mechanism by which the nucleus accumbens gates imput from the amygdala and hippocampus appears to be controlled by noradrenaline-dopamine*

interactions. Stimulation of β-receptors innervated by the LC checks the imput of hippocampal information into the VTA, an effect which is blocked by β but not α-receptor antagonists (Unemoto et al., 1985a and b). This 'gating' mechanism is indirect and additional to the direct 'gating' mechanism of hippocampal imput by the action of dopamine itself on D_2 receptors. Thus, noradrenergic activation closes the hippocampal 'gate' by indirect stimulation β-receptors and enhancement of D_2 neurotransmission; conversely, inhibiting β-receptors and D_2 receptors open the hippocampal 'gate'. The amygdala is a main processor of sensory and affective imput in the brain and relays information from cortical association areas to the VTA via the lateral hypothalamus. These projections may utilize somatostatin, neurotensin, or both. Though α-noradrenergic receptors are not involved with the activity of the hippocampal 'gate', there is evidence to suggest it has important interactions with a specific dopamine receptor subtype, the D_{Ai} receptor, in regulating the amygdaloid gate (Cools and Van Rossum, 1980; Cools, 1986). Thus, α-receptor antagonists close the amygdaloid 'gate' and suppress dopamine induced hyperactivity by systemic d-amphetamine. Notably, the effect of closing the amygdaloid gate and opening the hippocampal gate are functionally similar across a wide range of behaviours. Importantly, however, β-receptor antagonists, which open the hippocampal gate, only block d-amphetamine-induced hyperactivity when given before but not after d-amphetamine. This may explain why D_2 receptor antagonists inhibit the acquisition of incentive motivational learning but does not suppress its maintenance once established (Beninger, 1983). An open amygdaloid 'gate' is, therefore, essential for incentive motivational learning. This noradrenergic-dopamine coupling (the 'seesaw' hypothesis) has been used to explain other important phenomena. For instance, in the stressed animal or with the presentation of a novel environment or stimulus, the 'seesaw' II state is achieved - the amygdaloid gate is open (and the hippocampal gate closed), there is high noradrenaline release, and incentive motivational learning is maximal; in contrast, the well habituated animal is in 'seesaw' state type I - an open hippocampal (but closed amygdaloid) gate, there is little or no noradrenaline release, and maintenance of responding is favoured. Nicotinic cholinergic afferents from the dorsolateral tegmental nucleus and the ventral parabrachial nucleus may also activate A10 cells (Gould et al., 1989). Peptides (e.g. enkepahalins) and other neuromodulators such substance P may be important in regulating stress-induced VTA activation. The various opioids are indirect stimulants of dopamine and each has a specific

mechanism of activation by acting on separate groups of neurones; at low doses, enkephalin injection onto the VTA enhances conditioned place preference and self-administration (Kelley et al., 1989).

The first-order efferents from the nucleus accumbens (orange) are GABA containing neurones whose innervation includes the VTA, lateral habenula (LH), and the ventral pallidum (VP). GABA stimulation of different regions of the VTA produces behaviourally distinct responses. For example, while GABAergic stimulation of the anterior VTA leads to sedation and GABA antagonism at the same site locomotion; stimulation of the caudal VTA produces locomotion, aggression, and feeding in satiated rats (Arnt and Scheel-Kruger, 1979; Stinus et al., 1982). The mechanism by which specific behaviours are selected in response to 'gated' stimuli from the VTA-nucleus accumbens axis remains poorly understood. The ventral pallidum is a critical output pathway as lesioning it with ibotenic acid blocks amphetamine-induced locomotion (Swerdlow et al., 1986) and the reinforcing effects of cocaine and heroin (Hubner and Koob, 1990). The habenula may be involved with integrating the outputs of the dorsal and ventral straitum (Thornton et al., 1987) by serving as a relay centre from the basal ganglia and other limbic areas to the VTA.

The other landmarks in the diagram are: *C-P (Caudate-putamen), AC (anterior commisure, SNr (Substantia Nigra); PAG (Periacqueductal grey), OT (olfactory tract), and Cer (cerebellum). $GABA_A$ receptor complexes are coloured pale blue. Dopaminergic fibres running from the VTA to the nucleus accumbens are shown in red.*

The diagram is by courtesy of George Koob (1992) and Elsevier Science Publishers Ltd.

6. Pharmacology of 5-HT_3 receptors

6.1. Discovery, identification, and nature of the 5-HT_3 receptor

In studies on the sympathetic control of the cardiovascular system, Fozard and colleagues (Fozard & Mwaluko, 1976; Fozard & Mobarok-Ali, 1978) found that 5-HT induced noradrenaline release which was attenuated by (-) cocaine at non-5HT_1/5-HT_2 sites but not by methysergide. This suggested the presence of a novel subtype of 5-HT receptor, now known as the 5-HT_3 receptor. Classically, responses mediated by the 5-HT_3 receptor are: (a) unaffected by ketanserin (a selective 5-HT_2 blocker), methysergide (a non-selective 5-HT_1 and possibly 5-HT_2 antagonist), and methiothepin (a non-specific 5-HT_1, 5-HT_2 and perhaps 5-HT_{1D} antagonist); (b) stimulated by 2-methyl-5-HT, a 5-HT_3 agonist; (c) selectively attenuated by (-) cocaine and synthesized 5-HT_3 receptor antagonists such as MDL 72222 or ICS 205-930 (Bradley et al., 1986).

Peripherally, 5-HT_3 receptors are primarily located in the autonomic nervous system (Wallis, 1981; Fozard, 1984) where they may be involved in the modulation of inflammatory pain (Richardson et al., 1985). Both ligand binding (Kilpatrick et al., 1987; Watling, et al., 1988) and autoradiographic studies (Hoyer et al., 1989) show a wide distribution of 5-HT_3 receptors in the central nervous system. The highest concentrations are in the nucleus tractus solitarius (Pratt et al., 1989), the sensory processing areas of the spinal cord

(dorsal horn), the nuclei of the vagus and trigeminal nerves, the area postrema and the limbic system (Kilpatrick et al., 1987 and 1989; Higgins et al., 1989; Barnes et al., 1990a).

The 5-HT_3 receptor is a ligand gated ion channel. 5-HT_3 receptor activation either peripherally or centrally leads to a depolarization, whereby membrane conductance to sodium and potassium is increased. Studies of NG108-15 (Yakel et al., 1990) and N18 cells (Yang, 1990) show that the cations are differentially permeable to the membrane (according to size), suggesting a water-filled pore with an approximate diameter of 0.75 nm); in contrast, the membrane is relatively impervious to anions (Yang et al., 1992). Interestingly, 5-HT_3 receptors have been cloned from NCB-20 neuroblastoma cells expressed in Xenopus oocytes (Maricq et al; 1991) which implies that the principal component of the channel is contained within a single subunit.

6.2. Impact of 5-HT_3 receptors antagonists on normal human behaviour

In preliminary investigations, Orwin and Fozard (1986) found that systemic injection of 0.3 mg/kg of MDL 72222, a selective 5-HT_3 receptor antagonist, inhibited the flare response produced by intradermal injection of 5-HT; additionally, the personal experience of Fozard who participated in the study was that "substantial blockade of peripheral 5-HT_3 receptors does not result in overt (behavioural) pharmacological activity" (Fozard, 1989). The

more compelling evidence is, however, that for the last few years 5-HT_3 receptor antagonists have been in widespread use world-wide for the treatment of post-operative nausea and vomiting, and emesis induced by chemotherapy. To date, there have been no reports of significant effects on normal human behaviour. This is consistent with the finding that 5-HT_3 antagonists have no discernible effects on normal animal behaviour (Costall et al., 1990).

6.3. Modification of brain dopamine function by 5-HT_3 receptors antagonists

The role of 5-HT in the moderation of mesolimbic activity though intensively investigated remains imperfectly understood. Before the advent of 5-HT_3 receptor antagonists, microinjection of 5-HT into the nucleus accumbens was shown to block dopamine or amphetamine-induced hyperactivity (Costall et al., 1976; Pycock et al., 1978; Jones et al., 1981). Chemical lesions of 5-HT fibres in the nucleus accumbens by 5,7 dihydroxytryptamine leads to a rise in both spontaneous and amphetamine-induced hyperactivity (Carter and Pycock, 1979; Lyness and Moore, 1981), and ablation of the median raphe nucleus increases dopamine-induced hyperactivity (Costall et al., 1976). It was therefore postulated, quite simply, that 5-HT exerted inhibitory control on dopaminergic function, and that drugs which antagonized 5-HT would facilitate locomotion. However, it soon became clear that such a simple explanation of 5-HT/dopamine interactions could not be supported. For instance, administration of drugs with varying

degrees of antagonism at 5-HT_1 and 5-HT_2 receptors (e.g. methysergide, cyproheptadine, metorgiline, and ritanserin) were shown to have little effect on locomotor activity at physiological doses and to impair motion only at high, and possibly non-specific, doses (Costall et al., 1988). In addition, the impact of injecting 5-HT into the nucleus accumbens on spontaneous locomotor activity remains variable: ineffectiveness, enhancement, and reduction have all been reported (Jackson et al., 1975; Pijenenburg et al., 1975; Costall et al., 1979; Makunjuola et al., 1980). It was, therefore, evident that 5-HT control of mesolimbic dopamine function was complex (as there was evidence for both facilitation and inhibition), and that important missing links confounded our understanding. Recently, the landmark discovery of a new subtype of 5-HT receptor (the 5-HT_3 receptor) has been of considerable scientific interest as it promises to be one of the essential clues to elucidating the nature of central 5-HT/dopamine interactions.

In a series of classical experiments, the ability of 5-HT_3 receptor antagonists to oppose mesolimbic dopaminergic activity has been demonstrated using three paradigms. Firstly, hyperactivity induced by the injection of dopamine into the nucleus accumbens or ventral tegmental area (VTA) of the rat or marmoset was blocked by the 5-HT_3 receptor antagonist GR 38032F, ondansetron (Costall et al., 1987). This blockade was comparatively greater than that achieved by the D_2 receptor blocker, fluphenazine. In addition, fluphenazine, unlike ondansetron, reduced activity to below baseline levels and lead to *'rebound'* hyperactivity when it was withdrawn (Costall et al., 1988).

Also, unilateral injection of dopamine infusion into amygdala stimulates hyperactivity (Bradbury et al., 1985) which can be attenuated by ondansetron or fluphenazine given either peripherally or injected into the same or contralateral side as the dopamine infusion (Costall et al., 1987). This suggests a role for the amygdala in the regulation of dopamine-induced hyperactivity and of 5-HT on inter-hemispheric control of limbic activity. Curiously, at high doses (100 μg/kg to 1000 μg/kg i.p. and bd.) the ability of the 5-HT_3 receptor antagonists ondansetron and ICS205-930 to antagonize dopamine-induced hyperactivity is lost (Costall et al., 1990). While there is, at present, no satisfactory explanation for this non-linear dose response pattern, it is important to note that this property is not shared by the newly developed selective 5-HT_3 receptor antagonist, GR 68775C (Costall and Naylor, 1992).

In the second model, acute micro-injection of amphetamine (which causes the release of endogenous dopamine) into the nucleus accumbens of the rat leads to hyperactivity which is blocked by peripheral administration or infusion of ondansetron into the nucleus accumbens (Butler et al., 1988). Amphetamine-induced hyperactivity is facilitated by administration of 2-methyl-5-HT (Costall et al., 1987). Interestingly, the 5-HT_3 antagonist, ondansetron, has been shown to block *CPP* to amphetamine (Van der Hoek et al, 1989), and MDL 72222 attenuates CPP to the amphetamine-like substance, MDMA (Bilsky and Reid, 1991); in gainsay, Carboni et al (1989) were unable to obtain a similar effect using a similar dose (0.03 mg/kg) of two other 5-HT_3 antagonists, tropisetron and MDL 72222 (Diagram 4). The present thesis

includes the first investigation of the effect of 5-HT_3 receptor blockade (using GR 38032F and GR 68775C) on amphetamine-induced behaviour in humans.

Diagram 4

INHIBITION OF CONDITIONED PLACE PREFERENCE BY 5-HT_3 RECEPTOR ANTAGONISTS

Drug of Abuse	5-HT_3 Antagonist	Attenuation of CPP?	Reference
Morphine	Tropisetron (0.03 mg/kg)	Yes	Carboni et al, 1988
	MDL 72222 (0.03 mg/kg)	Yes	Carboni et al, 1988
	Ondansetron (0.01 mg/kg)	Yes	Higgins et al, 1992
Nicotine	Tropisetron (0.03 mg/kg)	Yes	Carboni et al, 1988
	MDL 72222 (0.03 mg/kg)	Yes	Carboni et al, 1988
Amphetamine	Tropisetron (0.03 mg/kg)	No	Carboni et al, 1988
	MDL 72222 (0.03 mg/kg)	No	Carboni et al, 1988
	Ondansetron (0.03 mg/kg)	Yes	Van der Hoek et al, 1989
MDMA	MDL 72222 (0.03 mg/kg)	Yes	Bilsky & Reid, 1991

In the third paradigm, locomotor hyperactivity induced by injection of 3µg per side of the neurokinin agonist DiMe-C7 (which increases endogenous dopamine turnover {Eison et al., 1982}) into the VTA was attenuated by administration of 100 µg/kg sc. of GR 38032F (Hagan et al., 1987 and 1990).

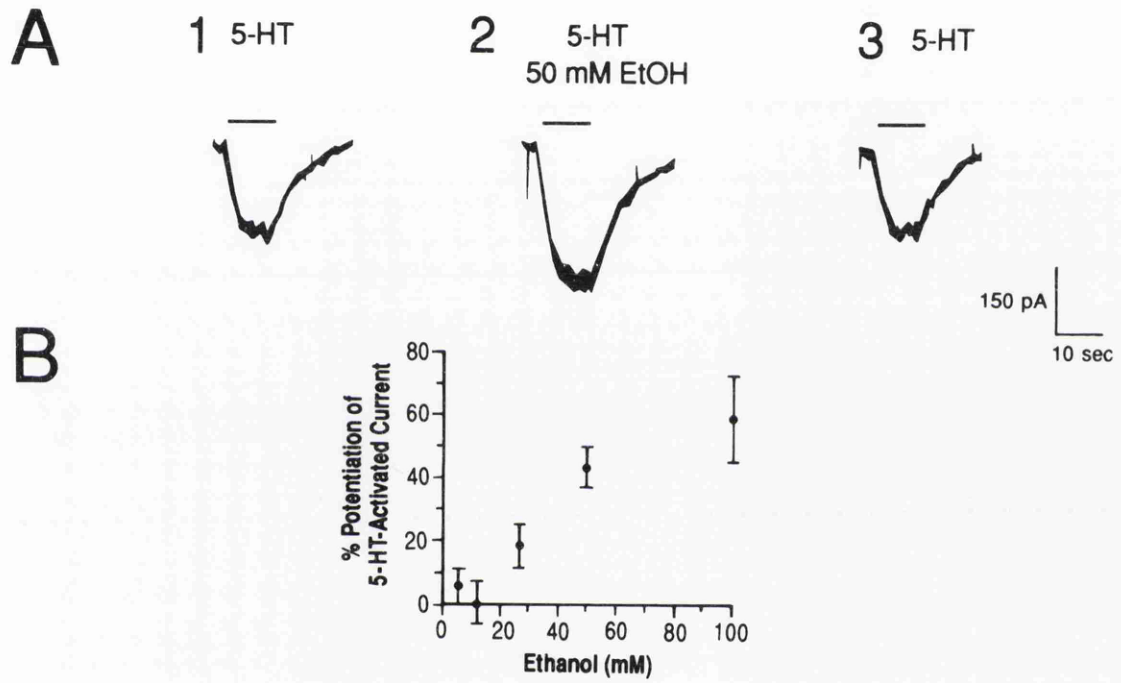
Interestingly, there is further evidence from in vivo studies that the 5-HT_3 receptor is intimately involved in the regulation of central dopamine release. For instance, intraventricular injection of the potent 5-HT_3 receptor agonists, 2-methyl-5-HT and 1-phenylbiguanide, into the nucleus accumbens of anaesthetised rats produces a dose-dependent increase in dopamine release (Jiang et al, 1990; Chen et al, 1991); also, the 5-HT_3 receptor antagonists, zacopride and ondansetron inhibit 1-phenylbiguanide-induced hyperactivity and decrease 3,4-dihydrophenylacetic acid (DOPAC) and homovanillic acid (HVA) levels when injected into freely moving rats (Chen et al, 1991).

6.4. 5-HT_3/dopamine interactions on alcohol-induced behaviour

From electrophysiological studies, the acute exposure to a small dose of ethanol increases 5-HT neurotransmission at excitatory 5-HT_3 receptors which facilitates central dopamine release {Diagram 5} (Lovinger and White, 1991). There are also excitatory effects at the nicotinic acetylcholine receptor, and activation of 5-HT_3 receptors stimulates the release of other neurotransmitters such as acetylcholine (Barnes et al., 1989), CCK (Paudice and Raiteri, 1991), noradrenaline, and GABA (Ropert and Guy, 1991). With intoxication, there is reduced neurotransmission at most excitatory receptors. Notably, there is inhibition of glutamate transmission at kainate and NMDA receptors, and impairment of voltage-sensitive Ca^{2+} channels; in contrast, there is increased activity at the inhibitory neurotransmitter, $GABA_A$. Dependence on ethanol causes

upregulation of NMDA and voltage sensitive Ca^{2+} channels and down regulation of $GABA_A$.

Diagram 5



Ethanol (EtOH) potentiation of the amplitude of 5-HT-activated current.

By courtesy of Lovinger and White (1991) and Neuroscience Letters.

It has been suggested that 5-HT_3 receptor activation modulates the reinforcing properties of alcohol by enhancing the release of dopamine (Blandina et al., 1988; Yoshimoto et al., 1992). Correspondingly, 5-HT_3 antagonists inhibit alcohol-induced dopamine release in the nucleus accumbens (Carboni et al., 1989; Costall et al., 1990; Wozniak et al., 1990), and decrease

alcohol consumption in a free-choice paradigm in rats (Sellers et al., 1988; Fadda et al., 1991; Knapp and Pohorecky, 1992). The ability of alcohol to act as a discriminative cue is attenuated by 5-HT_3 receptor blockade in pigeons (Grant and Barett, 1991) {diagram 6}. Importantly, environmental cues are also crucial to conditioning ethanol drinking behaviour and are powerful enough to evoke alcohol-seeking behaviour in the absence of alcohol (Samson and Li 1988a; Samson et al., 1988b; Samson and Grant, 1990). In addition, the intensity of the motivation to drink in humans is enhanced in the presence of the cue. If 5-HT_3 blockade can be shown to have a similar effect in humans, as in animals, it may open up new avenues for the treatment of alcohol abuse, and possibly of other addictive behaviours (Tricklebank, 1989).

Diagram 6

THE EFFECT OF 5-HT_3 RECEPTOR ANTAGONISTS ON ETHANOL CONSUMPTION/DISCRIMINATION - ANIMAL STUDIES

Test	Species	5-HT_3 Antagonist	Attenuation ?	Reference
Free choice in ethanol-preferring animals	Marmoset	Ondansetron (0.01 mg/kg bd)	Yes	Oakley et al, 1988
	Rat	Ondansetron (0.1 mg/kg bd)	Yes	Sellers et al, 1992
	Rat	Zacopride (5, 10 mg/kg bd)	Yes	Knapp & Pohorecky, 1992
	Rat (SP)	MDL 72222 (3-7 mg/kg bd)	Yes	Fadda et al, 1991
Drug discrimination	Pigeon	Tropisetron (0.1-0.56 mg/kg)	Yes	Grant & Barrett, 1991
		MDL 72222 (3-17 mg/kg bd)	Yes	Grant & Barrett, 1991
		Zacopride (0.56-1.7 mg/kg)	Yes	Grant & Barrett, 1991

6.5. Impact of 5-HT_3 receptor blockade on cognitive performance

Early experiments in rats have shown that the 5-HT_3 receptor antagonists, ondansetron and zacopride, can reverse scopolamine-induced deficits on the T-maze reinforced alternation task. Performance was, however, not improved in untreated rats (Barnes et al., 1990b). This reversal was attributed to facilitation of cholinergic transmission. Though 5-HT_3 receptors may mediate the release of acetylcholine in cortical tissue (Barnes et al., 1989), 5-HT_3 receptor antagonists, probably, only optimise cholinergic activity in undamaged neurones as they do not reverse the cognitive deficits produced by hemicholinium-3 lesions (Coughlan et al., 1991). Similar results have been obtained in the marmoset where ondansetron caused a dose-dependent decrease in the number of attempts to criteria in the object discrimination and reversal learning tasks (Domeney et al., 1991). From a preliminary study in humans, there is evidence to suggest that dosages between 0.25 mg and 1 mg bd. of ondansetron can improve performance in healthy 50 year olds with age-associated memory impairment on computerized tests of recall, new learning and reasoning (Crook and Larkin, 1991). Interestingly, 5-HT_3 antagonists do not correct benzodiazepine-induced impairment which suggests that neuronal pathways other than 5-HT_3-cholinergic interactions may mediate this effect.

6.6. 5-HT_3, and the effects of 5-HT_3 receptor antagonists on caloric and macronutrient intake following amphetamine

There is considerable evidence the 5-HT pathways in animals and humans are intimately involved in the regulation of feeding. Briefly, in animals, drugs which increase serotonin neurotransmission produce anorexia by: (a) increasing the availability of the 5-HT precursor, tryptophan (Latham and Blundell, 1979); (b) facilitating 5-HT release from nerve terminals {e.g. fenfluramine} (Samanin et al., 1980); (c) enhancing 5-HT activity such as quipazine and m-chlorophenylpiperazine, MCPP (Garattini, 1978) and (d) the administration of 5-HT itself to 'free-feeding' rats (Blundell and Latham, 1979) or rats with ventral medial hypothalamic (VMH) lesions (Bray and York, 1972). Further, the anorectic effects of fenfluramine can be reversed by 5-HT receptor blockade (Blundell and Latham, 1980), and the selective 5-HT re-uptake blockers, sertraline and fluoxetine, reduce food intake (Lucki et al., 1988; Clifton et al., 1989 respectively). Serotonergic mechanism also influence macronutrient intake. In particular, protein intake appears to be regulated by the balance between tryptophan and neutral amino acids (Anderson, 1979). In Wenling rats receiving fenfluramine or fluoxetine, both of which increase serotonin neurotransmission, the relative amount of protein ingested increased while total caloric and carbohydrate intake decreased (Wurtman and Wurtman, 1977 and 1979); conversely, serotonin depletion by intravenous ρ-chloro-phenylalanine or intraventricular 5,7-dihydroxytryptamine leads to a reduction in protein consumption. In addition, d-fenfluramine in both obese and normal

weight humans reduces caloric, carbohydrate and fat intake while sparing protein consumption {(Wurtman, 1981; Wurtman et al., 1982; Wurtman et al., 1985, McGuirk et al., 1991) and (Goodall et al., 1992)}. There are, however, some less definitive results on normal weight subjects (Blundell and Rodgers, 1980; Hill and Blundell, 1986).

While experiments using relatively specific agonists and antagonists at 5-HT receptors have implicated various subtypes of 5-HT_1 and 5-HT_2 in the regulation of feeding, the evidence for a central role for 5-HT_3 receptors requires further investigation. While some studies in rats have failed to demonstrate an effect on caloric intake in 'free-feeding rats' (Hutson et al., 1988; Kennett and Curzon, 1988; Dourish et al., 1989; Neill and Cooper, 1989); in contrast, others have shown a small reduction in feeding time over a narrow range of dosages (Van der Hoek and Cooper, 1990a; Fletcher and Davies, 1990). The findings of 5-HT agonists on feeding are hard to interpret. For examples, the 5-HT_3 agonists, 1,3 phenylbiguanide and 2-methyl-5-HT, decreased food intake when given i.p.(Kennett and Grewal, 1992; Simansky et al., 1991). This effect was not inhibited by 5-HT_3 receptor antagonists and may, therefore, be mediated by non 5-HT_3 mechanisms. Curiously, 2-methyl-5-HT was without effect when administered i.c.v. (Simansky et al., 1991). To date, there has only been one experiment on the effects of the 5-HT_3 receptor antagonist, ondansetron, on amphetamine-induced anorexia. This study found ondansetron increased sweetened mesh intake at certain dosages (30 μg and 100 μg) while reducing sucrose ingestion at 10 μg and 30

μg. Ondansetron also augmented the anorectic effects of amphetamine (Cooper et al., 1993). These results are hard to interpret because: (a) the size of the effect is small and may not be biologically relevant (although it is statistically significant), and (b) the direction of change is contradictory between the two paradigms. If these results are replicated and proven to be germane it does raise certain intriguing possibilities. These include: (i) amphetamine-induced anorexia is, at least in part, mediated by the facilitation of serotonergic transmission; (ii) ondansetron somehow regulates the on/off switch for the expression of anorexia, perhaps via cognitive processes; and (iii) ondansetron may, under certain conditions, enhance dopaminergic neurotransmission. The present thesis includes the first study of the 5-HT_3 receptor antagonist, GR 68775, on the anorectic properties of amphetamine in humans.

7. Objectives of the thesis

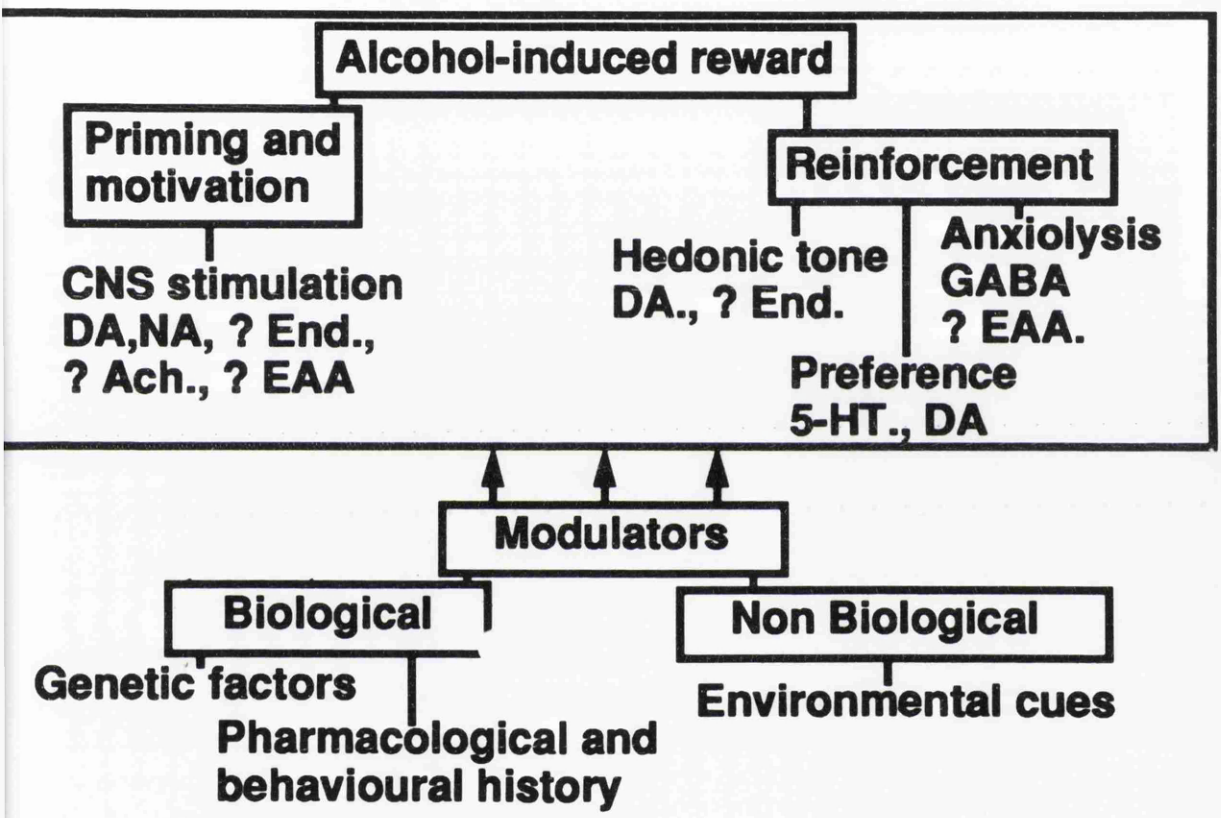
The present thesis contains the first laboratory experiments in humans designed to assess the impact of 5-HT_3 receptor blockade on the subjective and behavioural effects of alcohol and amphetamine. I have chosen to study these particular drugs of abuse because alcohol facilitates dopamine neurotransmission indirectly by increased 5-HT activity at 5-HT_3 receptors, and amphetamine causes the direct release of dopamine from nerve terminals. My hypothesis is that in humans, like animals, 5-HT_3 receptor blockade will attenuate dopamine-mediated subjective positive effects and behaviours induced by administration of these drugs of abuse. Specifically, I shall

investigate the effects of 5-HT_3 receptor blockade on subjective positive mood induced by alcohol and amphetamine. I shall also investigate whether the effects of 5-HT_3 receptor blockade on amphetamine-induced changes in cognitive performance, and caloric and macronutrient intake are inhibitory or enhancing.

8. Some practical considerations in the adaptation of animal studies into the design of human experiments

From animal studies, it is clear that both alcohol and amphetamine exert their rewarding effects via a range of neurobiological pathways and transmitters. *Priming* is a function of general central nervous system (CNS) stimulation. Reinforcement involves a complex interplay of both neurobiological and environmental systems. Key features of the reinforcement process such as increased hedonic tone, preference, and particularly for alcohol - the relief of anxiety - which at first sight appear to be mediated by diverse variety of neurobiological pathways, and environmental pressures such as experience and exposure, are thought to be expressed via a common final neuronal pathway mediated by dopamine. Diagram 7 below shows the contributions nature and nurture to the expression of alcohol-induced motivation and reinforcement.

***Diagram** 7: shows the contributions of the neurochemical pathways that mediate the impact of both biological and environmental cues on reinforcement, priming, and motivation following acute alcohol intake.*



Key: Dopamine (DA), endorphins (End), acetylcholine (Ach), excitatory amino acids (EAA), noradrenaline (NA), gamma-aminobutyric acid (GABA), serotonin (5-HT).

In humans, it is difficult to use operational measurements, as in animals, to quantify the reinforcing value of a drug of abuse. Hedonic tone is an important (Engel, 1977; Solomon, 1974; Stolerman, 1992), but not exclusive (Wise and Bozarth, 1987), facet of reinforcement since many drugs of abuse are dysphoric on first presentation yet self-administration can eventually occur. In animals, it has been suggested that positive hedonic tone increases the strength of reinforcement and sensitivity to the effects of the reinforcer. Anhedonia has the opposite consequence (Solomon and Corbitt, 1974; Carr et al., 1989; Stolerman, 1992; Willner et al., 1992). Chronic Mild Stress (CMS) has been shown to be a workable animal model of anhedonia which reduces the sensitivity of reinforcement. Trained animals after two weeks of CMS showed less preference for environments associated with one of the following reinforcers - 0.7% and 3.4% sucrose, food pellets, amphetamine (0.5-1.0 mg/kg), or morphine (0.7 mg/kg) - than non stressed animals. In addition, animals exposed to CMS show reduced sensitivity to the reinforcing effects of the D_2/D_3 agonist quinpirole (Willner et al., 1992). This appears to be homologous to the Solomon and Corbitt's (1974) model of hedonic tone and drug administration in humans. They suggested that in non dependent individuals, positive subjective well being (including hedonic tone), which may include pleasure, is directly related to the strength of the desire or urge to self-administer the reinforcer. In humans, the positive subjective effects on mood of reinforcers can be readily measured by standardized rating scales such as the Profile of Mood States questionnaire, POMS (McNair et al., 1971), or by simple visual analogue scales (VAS) such as the Bond-Lader Scale (Bond

and Lader, 1974) which are easier to use and sensitive to drug effects; however, VAS may be more prone to variability in the ratings.

In addition, I have adopted some of the lessons on conditioning from *CPP* in animals into my experiments in humans. In this paradigm, *familiarization*, whereby animals are pre-exposed to the test environment and conditions, is an important prerequisite as it reduces novelty. Similarly, in my experiments, subjects are *familiarized* with the test room and experiment measures the day before the onset of testing. The time spent during the *familiarization* is limited to about half of that for the test paradigm. This is because excessive exposure without the presentation of the reinforcer may lead to *latent inhibition*, whereby the difficulty of achieving conditioning is increased. It is confirmed that subjects understand the adjectival description of the variables on the VAS and are allowed to practice. Subjects are also trained until they reach 'ceiling' scores on the computerized psychomotor tasks.

EXPERIMENT 1

Hypotheses and objectives

Like other drugs of abuse, alcohol possesses reinforcing properties which are critical to directing, controlling, and sustaining drug-seeking behaviour (Stolerman, 1992). Animal experiments suggest that dopaminergic pathways running from the ventral tegmental area to the nucleus accumbens play a crucial role in mediating the reinforcing effects of drugs of abuse (Imperato and Di Chiara 1986; Wise and Bozarth, 1987, Koob, 1992). Thus, acute administration of alcohol increases extracellular levels of dopamine in the nucleus accumbens (Gessa et al., 1985; Koob, 1992), and dopamine receptor antagonists decrease alcohol consumption in a number of behavioural paradigms (Samson et al., 1988). A recently described subtype of 5-HT receptor, the 5-HT_3 receptor, appears to modulate the effect of alcohol on dopamine-mediated reinforcement processes. For example, in rodents, 5-HT_3 receptor antagonists inhibit alcohol-induced dopamine release in the nucleus accumbens (Carboni et al., 1989; Costall et al., 1990; Wozniak et al., 1990). Also, in vitro studies show that alcohol potentiates the ability of 5-HT_3 receptors to increase cation conductance (Lovinger and White, 1991). In humans, it is difficult to quantify the reinforcing value of a drug of abuse directly. It is, however, generally accepted that reinforcement can be measured indirectly since it is related, though not exclusively dependent, on its ability to produce positive subjective effects (Wise and Bozarth, 1987; Stolerman, 1992) which are more readily assessed.

It has been suggested that 5-HT_3 antagonists may improve cognitive deficits induced by scopolamine in the T-maze reinforced alternation task in rats (Barnes, 1990b) and the marmoset (1991), presumably by facilitation of cholinergic neurotransmission (Barnes, 1990a). A preliminary study in humans found improvements in age-associated memory impairment and on computerized tests of recall, new learning, and reasoning following ondansetron treatment (Crook and Larkin, 1991). To date, there has been no study of 5-HT_3/alcohol interactions on cognitive performance.

My objectives are to investigate whether the 5-HT_3 antagonist, ondansetron: (a) decreases the positive subjective effects of alcohol, and hence, its reinforcing value; and (b) improves cognitive performance in a computerized task, or reverses alcohol-induced deficits.

Method

Subjects

Sixteen healthy male volunteers between the ages of 19 and 49 years (mean age 32.8 years) who were social drinkers (mean ± s.e.m alcohol intake in units/week 10.8 ± 1.6) were studied. One unit of alcohol as defined by the Royal College of Physicians (1987) is a standard, single, public house measure of alcohol (e.g. ½ a pint of lager, beer or normal strength cider, one glass of

wine, or one single measure, 1/6 of a gill, of a spirit such as whisky or gin). Subjects were recruited from individuals who had shown a willingness in the past to be involved in psychopharmacological studies. Each subject received a psychiatric interview, to exclude a history of mental illness or substance misuse, and was examined medically to confirm they were in good health. Subjects consented in writing to participate in the study. Written consent was also obtained from their family doctors. Permission to conduct this investigation was granted by the Oxford District Psychiatric Research Ethics Committee.

Laboratory

The study was carried out at the MRC Psychopharmacology Unit in a testing room approximately 10 feet x 12 feet in size. In one corner of the room there was a desk and chair, and above it, a video camera and recording equipment. Output from the camera was displayed on a monitor in the nurses'observation station two rooms away. Subjects were informed they were being observed by the camera as a safety precaution and to monitor completion of the experimental measures. Subjects knew the session was not being recorded on videotape. An easy sofa chair was placed in front of a Viglen 486 DX computer which was connected to a printer, and a clock was sited on the window sill. Subjects were instructed to sit where they liked and allowed to roam freely in the room. Also, they kept their own time for the periodic measurements during the experiment. The testing room contained fictional material and magazines which subjects were allowed to read. Communication

between subjects and the experimenter was restricted to the time of dosing, setting up the computer, and presentation and measurements of breath alcohol as described in the design.

Visual Analogue Scales (VAS)

Subjects rated their subjective feelings at specified time points (the details are given below) on ten VAS items by placing a mark on a 100 mm line anchored with the endpoints of 'Not at all' on the left hand side and 'Extremely'on the right. VAS were scored for each time point by measuring the distance between the left hand side endpoint and the subject's mark. The following six adjectival variables were derived from descriptions of factor scores on the *Profile of Mood States [POMS]* (McNair et al, 1971): *"energetic", "relaxed", "agreeable", "cheerful", "confident",* and *"clear-headed"*; the rest (*"content", "optimistic", "feel good",* and *"desire for a drink"*) were chosen on the basis of a previous open study on the subjective effects of alcohol (see appendix 1a for a sample of the VAS). POMS variables were chosen because this scale has been shown to be a reliable measure of the mood-inducing effects of psychoactive drugs (Lorr et al., 1962; Mirin et al., 1971) including alcohol (Nathan et al., 1970a and b). The presentation of mood measures as VAS has been shown to be sensitive to psychostimulant effects (Chait and Johanson, 1988; Fischman et al., 1990).

Computerized Simple Reaction Time (SRT)

The SRT is a well known and validated computerized tests for assessing the effects of psychoactive drugs on cognitive performance and motor function (for reviews see Fleishman, 1964; Hindmarsh, 1980; Janke, 1980; Smiley, 1987; Curran, 1988; Spiegel, 1989). In the SRT, the subject is shown an empty box in the centre of a computer screen as a warning stimulus. After a random delay of between 1 and 10 seconds, a target square is displayed within the box. The subject is required to respond to the display of the target box as quickly as possible by pressing the space bar. No control files are used as the random timing is generated internally. In this experiment, the SRT was configured to run for 3 minutes.

Design

Sixteen subjects received each of the following four treatments in a double-blind, randomized, Latin square design: (a) alcohol (580 ml of 3.6% alcohol content by volume of lager) + placebo ondansetron; (b) placebo alcohol (580 ml of alcohol-free lager) + placebo ondansetron; (c) placebo alcohol + ondansetron (4 mg) and (d) alcohol and ondansetron. There was a 4-7 day interval between treatments. Prior to testing, all subjects were *familiarized* with the test room conditions, and trained in the use of VAS as described previously. Possible differences in taste between the lager and alcohol-free lager were disguised by chilling and the addition of 20 ml of lime. The

effectiveness of this masking had been confirmed beforehand in a previous open study.

Subjects were studied after a 4 hour fast at 1400 hours when they received ondansetron or matching placebo. The drink of alcohol or placebo alcohol was given 60 minutes later. Breath alcohol concentration was measured 20 minutes following the drink with a Lion SD-2 alcometer (breathalyser). VAS on 100 mm lines were completed 20 and 10 minutes before ondansetron and then 3, 5, 10, 15, 20, 20, 35 minutes after the drink. Subjects were not allowed to refer to previously completed VAS. The SRT was carried just before the drink and after the last VAS.

Data analysis

The VAS rated 20 min. before ondansetron were used as baselines, and subsequent scores were subtracted from them. Subjects were categorized according to four groups: placebo and placebo, ondansetron and placebo alcohol, alcohol and placebo ondansetron, and alcohol and ondansetron. A three-way (for alcohol x ondansetron x time) repeated measures analysis of variance (ANOVA) was performed (table I). The mean of the change in the VAS scores from baseline was determined. Confidence intervals were derived for the difference in score between the groups; however, to minimize type I errors only those VAS variables on which there was a significant alcohol x ondansetron x time interaction at the 5% level from the ANOVA were

subjected to inter-group post-hoc paired t-tests (table II). Fig. I shows the change in mean scores from baseline were plotted against time for the following VAS all of which had significant interactions (at the 5% level or above) for alcohol x ondansetron x time: *"desire for a drink"*, *"optimistic"*, *"cheerful"*, and *"clear-headed"*; additionally, post-hoc one-way repeated measures ANOVA of alcohol and placebo ondansetron Vs alcohol and ondansetron at individual time points were carried out.

A paired t-test was used to compare mean breath alcohol measurements 20 min. post alcohol for the alcohol and placebo ondansetron Vs alcohol and ondansetron groups.

The mean SRT before the drink was used as the baseline and the post drink mean SRT was subtracted from it to derive the average change in reaction time. For the four groups, a one-way repeated measures ANOVA was performed.

Results

Table I shows that alcohol produced significant effects (alcohol x time interactions) on all VAS ratings except *'confident'*. The effects of alcohol were significantly attenuated by ondansetron (alcohol x ondansetron x time interactions) for 6 of the 10 VAS scales: *"cheerful"* (F=2.34; df=7,105; p=0.029); *"confident"* (F=3.70; df=7,105; p=0.001); *"energetic"* (F=2.92;

df=7,105; p=0.008); *"optimistic"* (F=2.54; df=7,105; p=0.018), *"content"* (F=2.91; df=7,105; p=0.008), and *"desire for a drink"* (F=6.92; df=7,105; p<0.0001). Ondansetron did not modify the effect of alcohol on the following measures: *"feel good"* (F=1.29; df=7,105; p=0.26); *"clear-headed"* (F=0.95; df=7,105; p=0.47) or *"relaxed"* (F=1.08, df=7,105; p=0.38). Ratings of *"agreeable"* showed a significant alcohol x ondansetron interaction (F=6.03; df=1,15; p=0.027) but the interaction between alcohol, ondansetron and time was not significant (F=0.90; df=7,105; p=0.51). Table II shows the inter-group comparisons and confidence intervals for mean change in visual analogue scales from baseline. From this it can be seen that compared to placebo, ondansetron by itself had no effect on any of the VAS ratings. No tests for carryover effects were significant at the 5% level (data not shown).

There was no effect of ondansetron on breath alcohol concentration, as measured by a Lion SD-2 alcometer (breathalyser), 20 min. after alcohol ingestion: (mean {s.e.m} concentration following alcohol and placebo, 30{2.0} mg/100 ml; following alcohol and ondansetron, 30{1.9} mg/100 ml).

The mean change {s.e.m} in SRT from baseline following the drink was as follows: placebo alcohol and placebo ondansetron 17.46 ± {13.48} milliseconds; placebo alcohol and ondansetron 9.33 ± {13.79} milliseconds; alcohol and placebo ondansetron 21.03 ± {11.24} milliseconds; alcohol and ondansetron 14.19 ± {7.2} milliseconds. There were no significant main effects

for alcohol F=0.12; df=1,2; p=0.72 or ondansetron F=0.41; df=1,2; p=0.52, or alcohol x ondansetron interactions F=0.001; df=3,60; p=0.95.

Discussion

These findings suggest that 5-HT_3 receptor blockade attenuates some of the subjective effects of alcohol on mood, and reduces the subjective desire to drink. The effect of ondansetron to decrease some of the pleasurable effects of alcohol may be correlated with a reduction in its reinforcing properties of alcohol but it is important to point that this was not measured directly. It may be that a reduction in the pleasurable effects of alcohol by ondansetron could decrease alcohol consumption in a free-choice paradigm. Interestingly, animal studies indicate that a decrease in the reinforcing effects of a drug does not necessarily lead to reduction in drug-seeking behaviour. Indeed, partial blockade of the reinforcing effects of other drugs of abuse such as amphetamine and cocaine have been shown to elicit the opposite response, as the animal works harder to overcome to obtain the reinforcer (Davis and Smith, 1975; De Wit and Wise, 1977).

It has been suggested that increased release of dopamine in the nucleus accumbens may play a critical role in mediating the reinforcing effects of drugs of abuse. If ondansetron does attenuate the reinforcing properties of alcohol in humans it is tempting to speculate that this effect could be due to blockade of

dopamine release in the nucleus accumbens (Carboni et al, 1989; Fadda et al, 1989). There is, however, no direct evidence for this proposal at present.

Importantly, the psychological effects of alcohol may involve neurochemical mechanisms other than alterations in 5-HT or dopaminergic activity. For example, the sedative and anxiolytic effects of alcohol have been linked to increased activity at $GABA_A$ synapses. In this respect it is of interest that ondansetron did not significantly reduce the effect of alcohol on subjective ratings of *"clear-headed"* and *"relaxed"*. However, at present, it is not possible to link with certainty any particular psychological effect of alcohol with a specific neurotransmitter system. For example, it is worth noting that although ondansetron appeared to attenuate several of the pleasurable effects of alcohol it did not significantly alter ratings of *"feel good"*. Currently, there is no obvious explanation for this discrepancy.

It is important to consider that ondansetron may have altered the gastric absorption of alcohol. In gainsay, there was no effect of alcohol on breath alcohol concentration and a previous pharmacokinetic study using a much larger dose of ondansetron (24 mg) found no effect on blood alcohol levels (Preston C, personal communication). The lack of a main effect of alcohol on SRT reflects, perhaps, that impairment of performance is modest at low doses of alcohol. It is also possible that small deficits in performance were masked by notable inter-subject variability. In addition, there was no evidence that the small deficit observed was reversed by ondansetron suggesting that non $5\text{-}HT_3$

interactions may mediate this effect. Further studies are needed to confirm and extend the current preliminary findings, and to assess the effect of 5-HT_3 receptor antagonists on drinking behaviour.

Conclusions

1. Ondansetron attenuates some alcohol-induced mood changes and the desire to consume alcohol. I have hypothesized that this effect of ondansetron is correlated with a decrease in the reinforcing properties of the drug, though this was not measured directly. It is of interest that a preliminary clinical trial by Sellers et al. (1992) found that six weeks of treatment with ondansetron, albeit at a lower dosage (0.25 mg bd.), reduced the alcohol intake of heavy drinkers; however, this effect was only apparent after the drug was stopped. It also highlighted the suggestion from animal studies that the dose-response characteristics of ondansetron might be non-linear (Costall et al., 1987) Importantly, however, in a recent human laboratory study of similar design to this experiment, Jasinski et al. (1992) found that both 0.25 mg and 2 mg iv of ondansetron attenuated the subjective feeling of *"how much like the cocaine (in this case, cocaine was the reinforcer)?"*; the effect was much greater at the higher dose. It is tempting to speculate that the higher intravenous dose (2 mg) may be roughly equivalent to a dosage of about 4 mg orally as approximately 50-60% of the oral preparation is bioavailable (Pritchard, 1992). If it turns out that lower doses are more effective in clinical trials than human laboratory experiments it raises the interesting possibility that the therapeutic window of

the drug may be differentially sensitive to subjective and behavioural effects, and possibly, paradigm; indeed, there is some recent evidence to support this hypothesis (Cooper et al., 1993). Further clinical trials are needed to confirm the efficacy of ondansetron to diminish drinking in alcohol abusers. In addition, there is a need to investigate the dose-response characteristics to the psychological effects of ondansetron in humans. These investigations are, however, outside the scope of the present thesis. It is also possible that future clinical trials will be conducted using second generation 5-HT_3 antagonists such as GR 68775 which apparently have linear dose-response characteristics (Costall et al., 1992). Experiment 2 is a replication of the present study using a simpler design and a different group of subjects.

2. While there was no significant difference in breath alcohol readings after alcohol and placebo compared with alcohol and ondansetron, this measurement can only be reliably attained on the breathalyser 20 min. post alcohol. Thus, this measurement may simply reflect 'ceiling' levels following absorption. It was, therefore, not possible to exclude the possibility that there might have been differences in serum alcohol concentration between the two groups earlier or across the time course of the experiment. To exclude this possible confounding variable I conducted a pharmacokinetic study (see experiment 3).

EXPERIMENT 2

Objective

To confirm whether ondansetron attenuates some of the positive subjective effects of alcohol and the desire to drink as shown in experiment 1.

Method

Subjects

Twelve healthy male volunteers (mean age 29.8 years; range 20-48 years) who were social drinkers (mean alcohol intake in units/week {s.e.m}, 9.3 {1.2}) were studied. Recruitment and assessment of subjects to confirm there was no history of substance misuse or psychiatric disorder, and that they were in good physical health was as for experiment 1. Subjects gave written informed consent to participate in the study. Written consent was also obtained from their family doctors. Permission to conduct this study was provided by the Oxford District psychiatric Research Ethics Committee.

VAS

For this experiment, the most sensitive VAS measures to acute alcohol from experiment 1 were chosen. They were: *"agreeable"*, *"cheerful"*,

"clear-headed", *"confident"*, *"content"*, *"optimistic"*, and *"desire for a drink"*. A new variable, *"buzz"*, was added to assess the immediate positive effects of alcohol ingestion, making a total of eight variables for the VAS. Subjects rated their subjective feelings at specified time points by placing a mark on a 100 mm line anchored with the endpoints of 'Not at all' on the left hand side and 'Extremely'on the right. VAS were scored for each time point by measuring the distance between the left hand side endpoint and the subject's mark.

Design

The laboratory setting was as in experiment 1. The *familiarization* procedure prior to testing, and the time interval between test days was as in experiment 1. In contrast to experiment 1 there was no computerized cognitive performance test. Each subject received the following two treatments in a double-blind, randomized, crossover design: (a) alcohol (580 ml of 3.6% alcohol content by volume of lager) and ondansetron (4 mg orally); (b) alcohol and placebo ondansetron.

Subjects were studied after a four hour fast at 1400h when they received either ondansetron or matching placebo. Alcohol was given 60 min. later. VAS on 100 mm lines were completed 20 and 10 min. before ondansetron or placebo ondansetron, just before alcohol (time 0), and then 3, 5, 10, 15, 20, and 35 min after alcohol. Subjects were not allowed to refer to

previously completed VAS. Breath alcohol concentration was measured 20 min after alcohol with a Lion SD-2 alcometer (breathalyser).

Data analysis

For the two treatment groups, the median and interquartile range of the VAS rated 10 min. before ondansetron were compared with those scored just before alcohol (time 0) using the Wilcoxon Signed-Rank test. As this period includes the time when subjects had received only ondansetron or matching placebo, this comparison was used to determine if there was any independent effect of ondansetron. VAS scored post alcohol (i.e. after time 0) were subtracted from those rated just before the drink of alcohol (i.e. at time 0). Area under curve analysis (AUC) from baseline were calculated on the data. The median and interquartile ranges of the AUCs for the VAS scores were determined for the treatment groups and compared using Wilcoxon Signed-Rank test. The mean breath alcohol concentration 20 min. post alcohol was determined for both groups and compared using a paired t-test.

Results

There were no independent effects of ondansetron on any of the VAS ratings (table III). Alcohol and ondansetron produced significantly lower ratings than alcohol and placebo on the following VAS ratings: *"agreeable"*, *"confident"*, *"content"*, *"optimistic"* and *"desire for a drink"*. There was no

significant difference between the two groups on: *"buzz"*, *"cheerful"*, *"clear-headed"*, and *"confident"* (table IV and fig. II). Non parametric statistical methods were used for these analyses because the data was not normally distributed.

There was no significant difference in mean alcohol concentration {s.e.m} following alcohol and placebo compared with alcohol and ondansetron; 27.1 ± {2.6} mg/ml and 30.00 ± {3.4} mg/ml respectively (t=1.0; df=11; p=0.33).

Conclusion

The pleasurable effects of alcohol, and particularly the desire or urge to drink, are important to its reinforcing properties. In a recent experiment, De Wit and Chutuape (1993) have confirmed my finding that the desire for alcohol increases over time (up to 60 min.) following oral administration of 0.25 to 0.5 g/kg of alcohol. In addition they showed that subjects were more likely to chose more alcohol over money if they had received an alcohol pre-load. It is, therefore, an importance that the major finding of these experiments is that ondansetron (4 mg orally) has been confirmed to reduce the *"desire for a drink"* following a small dose of alcohol. It is tempting to speculate that with a larger number of subjects ondansetron would have been shown to attenuate *"cheerful"* and *"confident"* and *"buzz"* as the AUC scores for the alcohol and ondansetron group were markedly less than those for subjects who

received alcohol and placebo. It is, however, also conceivable that the effect of ondansetron on alcohol-induced mood change on these measures lack robustness.

Future research outside the scope of this thesis will need to explore whether the ability of ondansetron to attenuate the *"desire to drink"* will lead to a measurable reduction in alcohol consumption in a laboratory setting. If this is successful, clinical trials in heavy drinkers would be warranted.

EXPERIMENT 3

Hypothesis and objective

In the previous experiments I found that a single oral dose of the 5-HT_3 antagonist, ondansetron (4 mg orally), attenuated the positive subjective effects of a small oral dose of alcohol in healthy male volunteers. While this effect is consistent with data from animal studies that 5-HT_3 receptor blockade can diminish the reinforcing properties of drugs of abuse (including alcohol), perhaps by opposition of dopaminergic function in the nucleus accumbens (Carboni et al., 1989; Wozniak et al., 1990; Yoshimoto et al., 1992), I could not exclude the possibility that ondansetron might have altered the bioavailabilty of alcohol. For instance, there is evidence that ondansetron inhibits gut motility (Talley et al., 1990). Thus, it is conceivable that ondansetron might have impaired the absorption of alcohol into the

bloodstream. In this experiment I investigated whether ondansetron impairs the absorption of a small dose of alcohol from the gut.

Method

Subjects

Eight male volunteers (mean age 31.1; range 20-51 years) who were in good physical health and who had no history of mental illness (including substance abuse) were studied.

Laboratory

Subjects were studied while sitting upright in bed in a clinical testing room (approximately 6 x 8 feet in dimension) equipped with cardiovascular monitoring equipment.

Design

Subjects were tested twice in a double-blind, randomized, cross-over fashion. After a 4 hour fast at 1400 h, an intravenous cannula was inserted into an arm vein and it was kept patent by flushing with 5 ml of Hepsal solution (heparin sodium 10 units/ml). Subjects then received ondansetron (4 mg) or matching placebo. Sixty minutes later they consumed a drink of alcohol (580

ml of 3.6% alcohol content by volume of lager + 20 ml of lime juice) over a period of 1-2 minutes. Venous blood samples for alcohol estimation were drawn from the indwelling cannula at 5 minute intervals following alcohol ingestion for 30 minutes. Serum alcohol concentration was measured by a standard gas chromatography method.

Breath alcohol concentration was measured using a Lion SD-2 alcometer 20, 25, and 30 min. post alcohol.

Data analysis

For the two treatment groups - alcohol and ondansetron Vs alcohol and placebo ondansetron - means and standard errors, and the 95% CI for the difference between the groups were calculated for serum alcohol levels 3, 5, 10, 15, 20, 25, and 30 min. post alcohol. In addition, a two-way repeated measures analysis of variance (ANOVA) was used to compare the groups.

For both treatment groups, means and standard errors of breath alcohol concentration 20, 25, and 30 min. post alcohol were calculated. The correlation between breath and serum alcohol concentration 20, 25, and 30 min. post alcohol for each treatment group was determined using Pearson's correlation coefficient.

Results

Table V shows the means and standard error, and 95% CI for the difference between the two treatment groups on serum alcohol concentration. Serum alcohol concentration rose swiftly over time following the drink of lager ($F=30.4$, $df=6,42$; $p<0.001$). There was, however, no main effect of ondansetron ($F=0.44$, $df=1,42$; $p=0.53$) and no interaction between the effects of ondansetron and time ($F=0.29$, $df=6,42p=0.93$) {see fig. III}.

Table VI shows the comparison in means and standard errors, and correlation coefficients 20, 25, and 30 min. post alcohol for breath and serum alcohol concentrations between the treatment groups. The overall correlation coefficient between serum and breath alcohol concentration for the: (a) alcohol and ondansetron group was 0.83; $p<0.0001$; (b) alcohol and placebo ondansetron group was 0.84; $p<0.0001$.

Conclusions

While there was good agreement between breath and serum alcohol concentration for both groups taking into account all the readings post alcohol, there was little concordance at individual time points. In addition, breath alcohol readings were always higher than serum alcohol measurements. This may be due to marked inter-subject variability on breath alcohol readings, or contamination of measurements from gaseous alcohol in the nasopharynx, or

both. Importantly, however, the serum alcohol measurements confirm that ondansetron does not alter the absorption of a small dose of alcohol into the blood stream. These results are consistent with a previous study that used larger doses of alcohol and ondansetron (24 mg) where again no effect of ondansetron on the pharmacokinetics of alcohol was apparent (Preston, C; personal communication). I, therefore, conclude that the ability of ondansetron to diminish some of the positive subjective effects of alcohol is unlikely to be due to changes in alcohol absorption from the gut.

EXPERIMENT 4

Hypotheses and objectives

There is considerable evidence that the reinforcing effects of amphetamine are mediated by dopaminergic pathways. For instance, rats can be trained to lever press for amphetamine (Pickens and Harris, 1968; Wise et al., 1976), an effect which is attenuated by the administration of the D_2 receptor antagonist, pimozide (Yokel and Wise, 1985). Although amphetamines also facilitate the release of adrenaline from nerve terminals, amphetamine-induced responding is not attenuated by adrenergic antagonists such as phenoxybenzamine and propanolol (for a review see Neilsen and Anderson, 1992). while amphetamine reliably produces *CPP* in animals (Leone and Di Chiara, 1987; White et al., 1987), CPP to amphetamine is blocked by D_2 receptor antagonists such as haloperidol (Mithani et al., 1986) and 6-OHDA

lesions of the nucleus accumbens (Roberts and Koob, 1982). Similarly, in humans, the psychostimulant properties of amphetamine are attenuated by pimozide (Silverstone et al., 1980). It is, therefore, of interest that, in animals, 5-HT_3 receptor antagonists have been shown to inhibit amphetamine-induced hyperactivity (Costall et al., 1987) and *CPP* (Van der Hoek, 1989) and other dopamine-mediated behaviours in some paradigms (Imperato and Angelucci, 1989; Van der Hoek, 1990b; Chen et al., 1991) although there have been contradictory findings (Carboni et al., 1989; Moser, 1992). As the reinforcing properties of amphetamine are correlated with its positive subjective effects (Stolerman, 1992) it is hypothesized that 5-HT_3 receptor antagonists would attenuate these mood changes in humans, thereby reducing its reinforcing value.

It is well established that pharmacological doses of amphetamine (2.5 mg to 30 mg orally) increase the speed of response, vigilance and attention on computerized tests (Spiegel 1979 and 1989; Harvey, 1987). These responses appear to be mediated by the enhanced release of dopamine and noradrenaline. Interestingly, 5-HT_3 receptor antagonists, which oppose mesolimbic dopamine activity and inhibit 5-HT neurotransmission, have been shown to improve learning and performance on memory dependent tasks in animals. Additionally, in humans, 5-HT_3 antagonists improve age-associated impairments, possibly by facilitation of cholinergic neurotransmission (Barnes 1989a and b; Crook and Larkin, 1991). To date, there has, however, been no study of the effects of 5-HT_3 antagonist/amphetamine interaction on cognitive performance.

In animals, *d*-amphetamine causes the release of both noradrenaline and dopamine (Carlsson, 1970). In addition, brain stimulation, lesioning, and behavioural studies in animals suggest that it is this increased catecholamine availability that is responsible for amphetamine's ability to suppress food intake. Anorexia induced by amphetamine (at a dose of 1.25 mg/Kg) and mazindol (7.5 mg/Kg) is blocked by lesions of the ventral noradrenergic bundle (Samanin et al, 1977), and disruption of catecholamine synthesis by alpha-methyl-para-tyrosine reduces food intake (Baez, 1974). Notably, amphetamine-induced anorexia is only partially attenuated by dopamine receptor blockade with α-flupenthixol at relatively high (Garattini and Samanin, 1976) but not at low dosages (Burridge and Blundell, 1979). Hence, it has been suggested that noradrenergic rather than dopamine stimulation may be more critical to maintaining anorexia following amphetamine in animals (Samanin et al., 1978; Samanin and Garattini, 1982). In humans, *d*-amphetamine also induces anorexia, an effect which is attenuated by the D_2 receptor antagonist, α-flupenthixol (Garattini and Samanin, 1976; and for a monograph see Silverstone, 1992). However, amphetamine-induced anorexia is not attenuated in humans by the noradrenergic blocking agent, thymoxamine (Goodall et al., 1987). Since 5-HT_3 receptor antagonists oppose mesolimbic dopamine function, it would be predicted that 5-HT_3 antagonists, which oppose mesolimbic dopamine activity, might attenuate the anorectic effects of amphetamine.

The present pilot investigation was the first human study of the effects of 5-HT_3 antagonists on amphetamine-induced behaviours. As there had been some contradictory findings from animal experiments, the objectives were to investigate whether there was any evidence that ondansetron could attenuate the ability of amphetamine to induce positive subjective mood and anorexia, and to study 5-HT_3 antagonist/amphetamine interactions on reaction time and vigilance. I planned to confirm my positive findings in a follow up study.

METHOD

Subjects

Nine subjects between the ages of 21 and 47 years (mean age 34.1 years) were screened by psychiatric interview and medical examination to confirm they had no history of mental illness (including substance misuse), and were in good physical health before they were entered into the study. Subjects gave informed written consent to participate in the study. Written permission was also obtained from their family doctors. Permission to conduct the study was granted by the Oxford District Psychiatric Research Ethics Committee.

Laboratory

Laboratory conditions were as described in previous experiments 1 and 2.

Rating Scales

Subjects rated their subjective feelings at specified time points on six visual analogue scales (VAS) items by placing a mark on a 100 mm line anchored with the endpoints of 'Not at all' on the left hand side and 'Extremely'on the right. VAS were scored for each time point by measuring the distance between the left hand side endpoint and the subject's mark. The adjectival variables for the VAS were derived from ratings which had been shown to be sensitive to the effects of amphetamine by other experimenters (Silverstone et al., 1968 and 1980; Jacobs et al., 1986). They were: *"hunger"*, "positive *mood"*, *"energy"*, *"alertness"*, *"restlessness"*, and *"irritability"*.

The subjects' report of overall subjective abnormality was rated on a three point scale. The points on the scale were: 0-normal, 1-slightly abnormal, light-headed, or "high", and 2-definitely abnormal, light-headed, or "high".

Computerized Tests

1. The simple reaction time (SRT) test used was as in experiment 1.

2. The modified Stroop colour naming task measures interference to the cognitive processing of mood congruent words. It is also a test of vigilance as individuals are required to name the colour of a word as soon as it is detected. Five different colours are presented in random order. Distraction is produced by asking a person to name the colour of a word with emotional significance

with respect to their mood state. For instance, an anxious person would be expected to spend more time processing threat related words such as "panic" (Stroop, 1935). In this experiment, the Stroop was modified such that the words generated by the programme fell into one of three groups: low-mood (e.g. downcast), elevated mood (e.g. happy), and neutral words (e.g. typewriter). There were 48 words in each group, making a total of 144, and the presentation of words was in a random fashion. The words were presented on screen for 15 milliseconds; half the words were covered by a string of characters of a certain colour (i.e. "masked"). Subjects were required to name the colour of the words if they were "unmasked", and of the masking string if they were covered. The time taken to name each colour was recorded (in milliseconds) using a voice activated microphone connected a computer fitted with a Labtender timer card. The Stroop has been reliably used as a measure of focused attention to nicotine-induced improvements (Wesnes and Warburton, 1978), difficulty in concentrating produced by atropine (Callaway and Band, 1958), and deficits caused by scopolamine (Wesness and Revell, 1984). It was expected that amphetamine would enhance Stroop performance, and possibly, increase selective attention to mood-elevating words. "Masking" of some words was adopted because there is some evidence that this increases the sensitivity of the test.

Systolic blood pressure

Measurements of systolic blood pressure (using a mercury sphygmomanometer) were taken by a blinded observer.

Design

Subjects received the following treatments in a double-blind, randomized, cross-over design: (1) placebo ondansetron and placebo amphetamine; (2) placebo ondansetron and amphetamine (15 mg); and (3) ondansetron (12 mg in total) and amphetamine. In the twenty four hour period prior to the testing, subjects received pre-loading with ondansetron or matching placebo (to obtain good serum levels of ondansetron) in two doses of 4 mg each every 12 hours, making a total of 8 mg. Subjects fasted overnight and received the third of three 4 mg doses of ondansetron or matching placebo at 09.00 h (baseline). Thus, although there was no ondansetron and placebo amphetamine group, independent effects of ondansetron were estimated by comparing baseline scores. At 09.30 h, thirty minutes post baseline, subjects received 15 mg of amphetamine or matching placebo. VAS were completed from baseline, and every 30 min. from 10.30 h to 12.30 h (i.e. from 1 to 3 hours following amphetamine or matching placebo). Subjects were not allowed to refer to previously completed VAS. In addition, at 12.30 h subjects assessed their overall subjective abnormality during the session. The timing of the blood pressure measurements were as for the VAS. The blood pressure measurement

at 0900 h was taken as the baseline reading and subsequent recordings were subtracted from this. The computerized tests were administered at baseline (0.900 h), and 2 hours and 4 hours following amphetamine or matching placebo. The Stroop was administered prior to the SRT. Test days were separated by a washout period of at least seven days.

Data analysis

Differences between test days in mean scores on the VAS were assessed using the Student's two-tailed paired t-test. Changes in the mean ratings for overall subjective abnormality were analyzed by the Wilcoxon's signed rank test for paired samples. Paired t-tests were used to compare mean differences in systolic blood pressure in the 1 h to 3 h period post amphetamine. On the computerized tests, paired t-tests were conducted on the mean change in time from baseline to 2 h and 4 h post-amphetamine. In the Stroop colour naming task, independent effects on the three lists (low mood, elevated mood, and neutral words) were sought using a three-way repeated measures analysis of variance (ANOVA). Descriptive statistics are given as mean ± s.e.m, except for the overall subjective abnormality scale which is defined by the median and range.

RESULTS

Visual analogue Scales

Amphetamine produced a decrease in self-ratings of hunger compared with placebo. There were significant differences between: (a) placebo and placebo, and (b) placebo ondansetron and amphetamine which peaked 2.5 h following amphetamine ($t = 2.46$; $df = 8$; $p<0.05$). Following ondansetron pre-treatment, however, the decrease in hunger produced by amphetamine was attenuated - there was a significant difference between: (i) placebo ondansetron and amphetamine, and (ii) ondansetron and amphetamine at 2.5 h ($t = 2.37$; $df = 8$; $p<0.05$). Baseline ratings of hunger were not altered by ondansetron pre-treatment (data not shown) {see fig. IV}. Amphetamine caused a small but statistically insignificant increase, which peaked at 2 h, in VAS for: (a) *"restlessness"* (mean change in placebo and placebo group 9.6 ± 2.4 mm Vs mean change in the placebo ondansetron and amphetamine group 20.7 ± 5.6 mm), and (b) *"irritability"* (mean change in placebo and placebo group 7.3 ± 2.4 mm Vs mean change in the placebo ondansetron and amphetamine group 10.6 ± 5.6 mm Vs mean change in ondansetron and amphetamine group 4.0 ± 4.9 mm). There were no statistically significant changes in the VAS for "positive *mood", "energy", or "alertness"* in the placebo ondansetron and amphetamine group compared with the placebo and placebo group. Nevertheless, there were small increases ratings of "positive *mood"* in the

placebo and amphetamine group (9.2 ± 6.0 mm) compared with the placebo + placebo group (5.3 ± 2.9 mm).

Overall subjective state

Amphetamine significantly increased ratings of overall subjective abnormality, and this increase was attenuated by ondansetron. The median score for the placebo ondansetron + amphetamine group was 2 (range 1-2); in contrast, median scores for the placebo + placebo group was 0.5, range 0-1 ($Z = 2.59$; $p<0.05$), and for the ondansetron + placebo amphetamine group the median was 1, range 0-2 ($Z = 2.12$; $p<0.05$) {see fig. V}.

Computerized tests

In the Stroop colour naming task, a three-way repeated measures ANOVA (i.e. main effect of word type x treatment group x time) was used to discover whether there was an independent effect of word type (from the three word lists). As there was no independent effect of word type ($F=2.32$; $df=2,144$; $p = 0.10$), all three different word types were analyzed together. There was a significant main effect of amphetamine ($F=6.67$; $df=2,144$; $p<0.02$). There was no significant difference in the placebo and placebo group from baseline in the mean time taken to name the colour from baseline at either 2 h (mean change -1.7 ± 11.2 ms) or 4 h (mean change + 21.4 ± 16.3 ms). In the placebo and amphetamine group the mean change from baseline was

significant at both the 2 h (mean change - 41.4 ± 11.6 ms; t = 2.51; df = 8; $p<0.02$) and at 4 h (mean change - 24.9 ± 16.0 ms; t = 2.16; df = 8; $p<0.05$). Pre-treatment with ondansetron did not attenuate the effects of amphetamine on the Stroop. Thus, the mean change from baseline in the ondansetron and amphetamine at 2h was (mean change - 37.4 ± 10.2 ms; t = 2.84; df = 8; $p<0.01$) and at 4h (mean change - 23.1 ± 11.0 ms; t = 2.07; df = 8; $p<0.05$) {see fig. VI}.

In the SRT, amphetamine caused a marked decrease from baseline at 2 h which reached statistical significance at 4 h. Thus at 4 h the mean change from baseline in the placebo + placebo group was 3.6 ± 6.1 ms; for the amphetamine + placebo ondansetron group it was - 16.3 ± 7.0 ms; t = 2.33; df = 8; $p<0.05$). Ondansetron did not attenuate the effect of amphetamine, and in the ondansetron + amphetamine group the mean change from baseline at 4 h was - 14.1 ± 6.8 ms; t = 2.06; df = 8; $p<0.05$) {see fig. VII).

Systolic blood pressure

Mean systolic blood pressure changed little for the placebo and placebo group during the study (mean increase + 0.27 ± 3.2 mm Hg). In the placebo and amphetamine group mean systolic pressure rose significantly compared with the placebo + placebo group (mean change + 6.6 ± 1.9 mm Hg; t = 3.3; df = 44; $p<0.002$). Ondansetron had no effect on this amphetamine-induced rise in systolic blood pressure with the mean increase in the ondansetron and

amphetamine group being + 6.4 ± 3.3 mm Hg, significantly greater than the placebo + placebo group ($t = 3.7$; $df = 44$; $p<0.001$) {see fig. VIII).

DISCUSSION

There are four methodological limitations to this preliminary study. Firstly, the dose of amphetamine used, 15 mg, appears to have been too low as it did not produce significant changes in VAS ratings of "positive *mood*", *"alertness"*, *"irritability"*, and *"energy"*, as is consistently seen with higher doses of between 20 mg and approximately 35 mg in normal human volunteers (Jacobs et al., 1986; Angrist et al., 1987). It is, therefore, not possible to determine whether or not these individual psychological changes would have been attenuated by ondansetron. Secondly, there was no ondansetron and placebo amphetamine group. Thus, independent effects of ondansetron could not be confidently excluded, and it was not possible to segregate its impact, if any, on the amphetamine and ondansetron group. Thirdly, serum levels of ondansetron and amphetamine were not measured. It is, therefore, not possible to exclude pharmocokinetic explanations for the effect of ondansetron on amphetamine-induced psychological changes. This is particularly pertinent as ondansetron has been shown to inhibit gut motility (Talley et al., 1990). It is, however, equally important to point out that in experiment 3 I was unable to uncover any evidence that 4 mg of ondansetron impaired the absorption a small dose of alcohol. This opinion is shared by an independent experimenter who found no significant effects of up to 24 mg of ondansetron on alcohol

absorption (Preston, C; personal communication). Fourthly, the predictive power of the study is hampered by the small number of subjects and modest but biologically relevant findings may go undetected.

The mechanism by which ondansetron attenuates amphetamine-induced anorexia is of particular relevance. Microinjection studies in rodents have suggested that the anorexic effect of amphetamine is mediated largely through facilitation of noradrenergic and dopaminergic neurotransmission (Blundell, 1985) in the lateral hypothalamus (Hoebel et al., 1989) and perhaps the nucleus accumbens (Cooper, 1991). Thus, the partial attenuation of amphetamine-induced anorexia by ondansetron is consistent with the effects on hunger of drugs which antagonize amphetamine-mediated dopamine release in animals (Samanin et al., 1977; Samanin and Garattini, 1982).

Amphetamine caused a significant elevation in ratings of overall subjective state, and a trend towards elevation of "positive *mood*". The biochemical mechanism underlying this change is uncertain. Nevertheless, in humans, amphetamine-induced mood elevation or arousal can be attenuated by dopaminergic but not noradrenergic antagonists (Gunne et al., 1972; Silverstone et al., 1980). This implies that amphetamine-induced mood elevation may be attributable to increases dopamine release. Animal studies of reward behaviour, probably the best model of mood elevation, have repeatedly shown that increased dopaminergic neurotransmission in the nucleus accumbens is crucial to the development, direction, and maintenance of reward

behaviour (Yokel and Wise 1976; Lyness et al., 1979; Stolerman, 1992). It is, however, important to point out that the present study did not produce significant increases in energy, alertness, or restlessness, all of which when induced by larger doses of amphetamine can be attenuated by dopamine receptor blockers (Silverstone et al., 1980; Jacobs and Silverstone, 1986). It is possible that the measurement of overall subjective state was either sensitive enough to pick up small amphetamine-induced changes in mood, or related to some other biochemical process. Repeating this experiment using a higher dose of amphetamine is critical to resolving this issue. Given that overall subjective state may be a global measure of amphetamine-induced mood, it is of importance that this was attenuated by ondansetron. This finding is in keeping with the results of a recent human experiment whereby ondansetron attenuated the *"rush"* of intravenous cocaine (Jasinski et al., 1992). Hence, as cocaine, like amphetamine, increases dopamine release in the limbic system, it is conceivable that the results from both these experiments represent evidence of attenuation of central dopamine release by ondansetron. This effect is also consistent with the finding in animals that 5-HT_3 antagonists inhibit amphetamine-induced hyperlocomotion (Costall et al., 1987). Notably some investigators have been unable to attenuate increased dopamine release following amphetamine with 5-HT_3 receptor antagonists (Carboni et al., 1989; Montgomery et al., 1993). However, the dosages of amphetamine used in these experiments were up to fifty times higher than in the study of Costall and co-workers (1987), the dosage of ondansetron was greater (and may have been subject to inactivity at the higher dose due to the non-linear dose-response

characteristics of ondansetron), and the route of drug administration was peripheral rather than systemic. It may be that 5-HT_3 antagonists can act either pre-synaptically to inhibit dopamine release or post-synaptically to attenuate the effects of increased dopamine release, or that 5-HT_3 antagonists may only inhibit reliably the action of drugs which increase dopamine neurotransmission transynaptically. Thus, it can be argued that the effects of drugs such as alcohol, morphine, and nicotine which indirectly facilitate dopaminergic neurotransmission transynaptically and which depend upon transmission at excitatory 5-HT_3 receptors, presumably located pre-synaptically, are those most likely to be inhibited by 5-HT_3 antagonists. In contrast, amphetamine and cocaine act by directly releasing dopamine from synaptic terminals or preventing its re-uptake. Dopamine cell firing is not increased and, therefore, amphetamine or cocaine mediated behaviours may not be affected by the activity of 5-HT_3 antagonists at presynaptic 5-HT_3 receptors. In sum, the precise biochemical mechanisms which underlie the effects of 5-HT_3 receptor antagonists on amphetamine-induced behaviour require clarification from further studies.

The findings of the present experiment are in keeping with the view that amphetamine improves performance in psychomotor tasks of speed and vigilance (Spiegel, 1989). It has been hypothesized that dopaminergic mechanisms may be involved in the control of these processes. Ondansetron had no obvious effect on cognitive performance and this is in keeping with a previous study in human volunteers (Hall and Ceuppens, 1991). Notably,

however, this study did not contain an ondansetron alone group which will be needed to confidently ascertain the effect of 5-HT3 receptor antagonism on cognitive performance in humans. Further studies will, therefore, have to include more sensitive tests of focused attention such as the rapid information processing task, and subjects should, perhaps, be trained to 'ceiling' levels before testing.

Amphetamine administration increases the release in the brain of noradrenaline, dopamine, and serotonin (Carlsson, 1980; Blundell, 1985; Hoebel et al., 1989). The finding from the present experiment of a rise in systolic blood pressure following amphetamine is in keeping with results from previous studies, one of which has shown that this rise in blood pressure can be blocked by noradrenergic receptor antagonism (Jacobs and Silverstone, 1986). It is, therefore, probable that increased systolic pressure following amphetamine is chiefly due to noradrenaline release. In the present experiment, amphetamine-induced increase in blood pressure was not attenuated by ondansetron. In general, animal studies have not supported a role for 5-HT_3 receptor antagonists on noradrenaline release. While one *in vitro* study has suggested that 5-HT_3 antagonists can inhibit noradrenaline release (Feuerstein and Hertting, 1986) no other study to date has replicated this finding. Support for a lack of effect of ondansetron on noradrenaline release also comes from animal models of morphine withdrawal. The behavioural syndrome of morphine withdrawal appears to be mediated by large increases in noradrenaline release within the brain (Rasmussen et al., 1990; Silverstone et al., 1992) and morphine

withdrawal can be attenuated by blockade of noradrenaline release with clonidine (Silverstone, 1992). In this respect it is noteworthy that ondansetron does not attenuate the morphine withdrawal syndrome (Higgins et al., 1991). Thus, the findings in the present experiment of a lack of effect of ondansetron on the increase in systolic blood pressure following amphetamine are in keeping with previous animal evidence of a lack of effect of 5-HT_3 antagonists on noradrenaline release.

CONCLUSIONS

1. Amphetamine-induced change in overall subjective state and anorexia are attenuated by ondansetron. The present experiment, therefore, supports the hypothesis that 5-HT_3 antagonists oppose the release of dopamine in the nucleus accumbens following amphetamine. Nevertheless, the psychological effects of amphetamine require further confirmation. In addition the anorectic affects of amphetamine with and without ondansetron need quantification using a test meal paradigm, wherein caloric intake and macronutrient selection can be measured directly.

2. Higher doses of amphetamine, greater than 15 mg orally, are needed to produce reliable elevation of mood.

3. Second generation 5-HT_3 antagonist such as GR 68775 with linear dose-response characteristics should be employed in subsequent studies.

4. The effects of 5-HT_3 antagonists on amphetamine-induced enhancement of reaction time and attention remain unclear. Further experiments should substitute the Stroop with, perhaps, a more sensitive measure of focused attention such as the rapid information processing task. Subjects should also be trained to achieve 'ceiling' levels of performance before formal testing.

EXPERIMENT 5

Hypotheses and objectives

The present study takes into consideration the lessons learnt from experiment 4 and explores the impact of pre-treatment with a novel second generation 5-HT_3 receptor antagonist, GR 68775, on the psychological effects of a higher dose of amphetamine (20 mg).

Specifically, the present study will assess the effects of GR 68775 on amphetamine-induced: (1) mood change - as measured by VAS and a global rating of subjective well being; (2) anorexia, and using a test meal, caloric intake and macronutrient selection; (3) enhancements in reaction time and vigilance

METHOD

GR 68755

GR 68755 is a second generation 5-HT_3 receptor antagonist. Animal models have shown GR 68755 to be active in situations which stimulate mesolimbic dopamine overactivity. GR 68755 inhibits locomotor hyperactivity in the rat caused by: (a) injection of DiMe-C7 (a neurokinin) into the ventral tegmental area (0.1 - 1000 μg/kg sc); (b) continuous infusion of dopamine into the nucleus accumbens (1 ng - 100 μg/kg ip), and (c) injection of amphetamine into the nucleus accumbens (100 ng intra accumbens). It has also been shown to improve cognitive performance in the marmoset at a dose of 0.1 ng to 1 mg/kg. Its putative advantages over ondansetron are that its dose-response characteristics are linear. To date, GR 68755 has been administered to 223 healthy volunteers and has been safe and well tolerated at all dose levels studied (up to 16 mg bd orally for 3.5 days and 2 mg orally for 27.5 days). Adverse events have been minor with headaches and constipation being the most commonly reported. These side-effects are typical of the class of 5-HT_3 antagonists. GR 68755 is rapidly absorbed into the blood stream after oral administration (peak absorption 45 min to 1 h) with a relatively short elimination half-life in comparison to ondansetron (GR 68755 1.6 - 2.7 h Vs ondansetron 3 - 4 h). Its bioavailability following an oral dose is similar to that of ondansetron 50-60%. Thus, GR 68755 appears to be most suitable for single rather than repeated dosing procedures. In this respect it is of interest that, in

humans, a single intravenous dose (2 mg) of GR 68755 reversed the scopolamine-induced cognitive effects 1 h and 3 h post dosing. In addition, a single oral dose (1 mg) of GR 68755 has been shown to antagonize the 5-HT mediated intradermal flare response with a duration of action of almost 10 h (Glaxo, unpublished data on file). On this evidence, I decided to use a single oral dose (2 mg) of GR 68755 to challenge the effects of *d*-amphetamine. To ensure that the antagonist, GR 68755, penetrated the central nervous system before the agonist challenge, GR 68755 (2 mg orally) was administered 30 min. before *d*-amphetamine.

Subjects

Twenty six male subjects between the ages of 18 and 45 years (mean age 28.9 years) were recruited. Before entering the study, they had a psychiatric interview, medical examination, and laboratory investigations (haematology, biochemistry, and urinalysis for drugs) to confirm that they had no history of mental illness (including substance misuse) and were in good physical health. Urine was taken for a drug screen after each test day. Consequently, two subjects found to be using cannabis during the study were replaced. One subject failed to attend for the fourth of four test days. Written informed consent was provided by the subjects and their family doctors before they were entered into the study. Permission to conduct the study was granted by the Oxford District Psychiatric Research Ethics Committee (see appendix 1b for details of volunteer screening, inclusion, exclusion and withdrawal criteria, and study day restrictions).

Laboratory

Laboratory conditions were as in experiment 1, 2, and 4. Subjects made no contact with the experimenters except for at the start of the experiment, re-setting the computer for the psychometric tests, and when the test meal was presented. To ensure their safety, and to confirm that the ratings were completed as scheduled, subjects were monitored on close-circuit television.

VAS(A)

The design of the VAS was as for the previous experiments. VAS(A) contained 16 adjectival variables which could be subdivided into four categories: mood/arousal (6), reinforcement (2), hunger (2), satiety (4), and suspiciousness (2). The ***mood/arousal*** scales were: *"confident"*, *"cheerful"*, *"mind-racing"*, *"alertness"*, *"restless"*, and *"lethargic"*; for ***reinforcement*** they were - *"feeling good"* and *"getting a buzz"*; for ***hunger*** they were - *"hungry"* and *"strong wish to eat"*; for ***satiety*** before the test meal they were *"I expect to find the meal satisfying"* and *"right now I could eat a large amount of food"*, and after the test meal - *"I found the meal satisfying"* and *"I feel full"*; for ***suspiciousness*** they were: *"feeling bothered"* and *"being treated fairly"*.

Global rating scale

The global rating scale measures overall subjective state on a five point scale: 0 - normal; 1 - slightly light-headed, restless, or speeded up; 2 - moderately light-headed, restless or speeded up; 3 - very light-headed, restless, or speeded up, 4 - extremely light-headed, restless, or speeded up. Space was provided at the bottom of the form for subjects to enter comments {appendix 1c}.

Choice of food items for the test meal and the test meal paradigm, satiety VAS(B), and the food preference questionnaires.

In the one week prior to testing, subjects were presented with a list of food items from which to chose the composition of their buffet style test meal {appendix 1d}. All food items were weighed to the nearest 0.1g before and after the meal. Macronutrient selection and caloric intake were calculated using specifically designed computer software (see Hill and Blundell, 1986 and acknowledgements).

VAS(B) contained items on the subjects' satiety before and after the test meal. The items scored before the meal were: *"I expect to find the meal satisfying"* and *"right now I could eat a large amount of food"*. After the meal, the scales were - *"I found the meal satisfying"* and *"I feel full"*.

Two questionnaires items were also completed before and after the meal. The first, the *forced choice questionnaire*, compelled subjects to chose an item from a selection of each of the following macronutrients - protein, fat, carbohydrate, or low energy items they would like to receive {appendix 1e}; specifically, the *forced choice questionnaire* examined preference for carbohydrate over protein items. The second, the *free choice questionnaire*, allowed subjects to chose whether or not they would like an item from any of these categories of macronutrients {appendix 1f}. Vegetarians or subjects restraining their diet would have been unable to fulfil the test meal criteria and were therefore not admitted into the study.

Computerized tests

1. The rationale for the SRT was described in experiment 1.

2. The Rapid Visual Information Processing Task (RVIPT) is a test of vigilance and attention. In this task subjects monitor digits which are presented sequentially on a computer screen at a rate of 100 per minute for 7.5 minutes. Subjects are instructed to detect and respond to targets of three consecutive odd or even digits as quickly as possible. Independent measures are made of both the speed and accuracy of decision making. The measures from the task were: Hits - correct responses within 600 ms; delayed hits - responses occurring 600 - 1200 ms after the target; false alarms - incorrect responses, and

reaction time for both hits and delayed responses. Measures were recorded for each 250-trial block.

Design

Subjects received each of the following four treatments in a double-blind, randomized, Latin square design: (a) *d*-amphetamine (20 mg oral) and placebo GR 68755; (b) placebo GR 68755 and placebo *d*-amphetamine; (c) placebo amphetamine and GR 68755 (2 mg) and (d) GR 68755 and *d*-amphetamine. There was a 4-7 day interval between treatments. Prior to testing, all subjects were *familiarized* with the test room conditions, and trained in the use of visual analogue scales as described previously.

Subjects arrived in the unit 0800 hours having fasted from 23.30 h the previous night. A specimen of urine was taken and subjects were allowed to settle in the test room for 30 min. At 08.30 h and 08.40 h baseline VAS(A) (i.e. for mood/arousal, reinforcement, hunger, and suspiciousness) were completed. Baseline RVIPT and SRT were carried out immediately afterwards. At 09.00 h GR 68755 or matching placebo was given, and thirty minutes later (09.30 h) *d*-amphetamine or its placebo. Subjects repeated their rating of the VAS(A) scales at baseline every 20 min. for the next 3 h (12.30 hours). Each set of VAS(A) were completed independently and subjects were not allowed to refer to previous ratings. After the 11.50 h VAS scales the computerized tests (RVIPT and SRT) were repeated. The computerized tests were chosen to

coincide with 2 h post *d*-amphetamine - the time at which peak amphetamine level has been previously recorded (Angrist et al., 1987). At 12.35 h subjects completed the pre-meal VAS(B) for satiety and the *forced* and *free choice food preference questionnaires*). The test meal was then brought in and subjects were instructed to ignore normal dietary restrictions and to eat as much as they wished. The time taken to eat the test meal was measured. Ten minutes after the meal, subjects filled in the post-meal VAS(B) and both post-meal food questionnaires. After this, subjects filled in the global rating questionnaire.

Data analysis

For the VAS measuring *mood/arousal, reinforcement, hunger, and suspiciousness* the baseline score was taken as the mean of the 08.30 h and 08.40 h ratings. Data recorded for these VAS after the dose of amphetamine or matching placebo were summarized by the weighted mean response over three hours. The weighted mean was calculated by dividing the area under the curve (AUC), obtained using the trapezoid method, by the length of time over which the measurement was recorded (3h). In addition, the weighted mean responses for the visual analogue scales were analyzed using analysis of covariance allowing for effects of due to subjects, periods and treatments with the baseline measurement as a covariate. Test for carryover were also performed. Estimates of treatment effect and 95% confidence intervals were calculated for the differences between *d*-amphetamine alone and placebo, GR 68755 and placebo, GR 68755 with

d-amphetamine and *d*-amphetamine alone, and GR 68755 with *d*-amphetamine and GR 68755 alone.

The global rating scale was analyzed by comparing treatments pairwise using the Wilcoxon's signed-rank test.

Pre and post-satiety VAS(B) were recorded before and after the test meal respectively. Responses for the food preference questionnaires were displayed as mean scores ± s.e.m. Differences pre and post the test meal were compared using a two-way repeated measures analysis of variance (ANOVA) design followed by paired t-tests. All food items were weighed before and after the test meal. Caloric intake and macronutrient selection was calculated using specifically designed computer software (see Hill and Blundell, 1986, and acknowledgements). Means and standard errors were also determined for this data. Caloric intake was analyzed using a repeated measures ANOVA design allowing for effects of subjects, periods and treatments. Estimates of treatment effects and 95% confidence intervals were calculated for the differences between *d*-amphetamine alone and placebo, GR 68755 and placebo, GR 68755 with *d*-amphetamine and *d*-amphetamine alone, and GR 68755 with *d*-amphetamine and GR 68755 alone. ANOVAs were also performed to detect differences between the treatment groups on macronutrient selection and preference for sweet or savoury foods.

For the computerized tests, only one measurement was taken pre-amphetamine or its placebo, and this was used as the baseline measurement. The tests were summarized as the mean number of correct hits per minute and mean reaction time on the RVIPT, and by the mean SRT. Weighted geometric means were calculated for the number of correct hits on the RVIPT, and the reaction times were log transformed. Estimates of treatment effect and 95% confidence intervals between amphetamine and placebo, GR 68755 and placebo, GR 68755 with amphetamine and amphetamine, GR 68755 with amphetamine and GR 68755 were performed.

RESULTS

Subjects

Twenty six subjects were recruited. Subject 8 had a positive urine test for cannabis on the second of four test days and, therefore, all data was excluded. Subject 19 had a positive test for cannabis on the post-study check and data for the fourth period was excluded. Subject 12 had data from only three periods. Subject 14 received GR 68755/placebo in two periods. There were, therefore, twenty four subjects in the placebo/placebo, amphetamine/placebo and GR 68755 and amphetamine groups, and twenty five subjects received GR 68755/placebo treatment.

VAS(A)

No tests for carryover effects were significant at the 5% level. There were no statistically significant differences between amphetamine alone and placebo, or GR 68755 with amphetamine and amphetamine alone for any of the VAS recorded. There were statistically significant effects of treatment, and for differences between: (i) amphetamine alone and placebo and (ii) GR 69755 with amphetamine and GR 68755 alone on the following visual analogue scales: *"confidence"*, *"cheerfulness"*, *"lethargy"*, *"alertness"*, *"mind racing"*, *"hunger"*, *"strong wish to eat"*, *"feeling good"* and *"getting a buzz"*. For *"restless"*, there was also a main treatment effect but the only statistically significant difference was between GR 68755 with amphetamine and GR 68755 alone. Subjects who received GR 68755 with amphetamine were more *"restless"* by 5 mm compared to those who got GR 68755 alone {95% Confidence Interval (CI) 2 to 8; df = 66; p=0.002}. There were no statistically significant treatment effects on *"feeling bothered"* and *"being treated fairly"*. See tables VII and VIII, and fig. IX.

Global Rating Scale

On the global rating scale, there was a significant difference between amphetamine alone and placebo {z = -3.51; p<0.001} and between GR 68755 with amphetamine and GR 68755 alone {z = -3.62; p<0.001}. There was,

however, no significant difference between either the GR 68755 and placebo group {z = -0.73; p=0.46}, or the GR 68755 with amphetamine and amphetamine alone group {z = -0.31; p=0.75}{see fig. X for the scores}.

Food preference VAS(B) and questionnaire, and the Test meal paradigm

For all the visual analogue scales for satiety, there was a statistically significant main effect of amphetamine. Subjects who received amphetamine alone or amphetamine with GR 68755 both expected to, and indeed, found the meal less satisfying than those who received placebo. There was no significant difference between subjects who received GR 68755 with amphetamine and those who got amphetamine alone on any of the VAS. Compared with placebo, subjects who received amphetamine or GR 68755 and amphetamine felt less able to *"eat a large amount of food"* before the meal, but after the meal, those who had amphetamine alone did not feel less full; however, no significant differences existed between the group which received GR 68755 and amphetamine and those who had amphetamine alone {table IX}.

There were no obvious preferences on any of the test paradigms on the *'forced'* and *'free food'* choice questionnaires and the data is displayed in table X.

Subjects who received amphetamine alone, or GR 68755 and amphetamine spent less time (in minutes) eating than the placebo group {(mean 14.2 ± 1.6 Vs 18.2 ± 1.5; 95% CI 1.5 to 6.4; t = 3.39, df = 23, p=0.003) and (mean 14.5 ± 1.1 Vs 18.2 ± 1.5; 95% CI 0.2 to 7.2; t = 2.2, df = 23, p=0.38) respectively}. There were no significant differences in the time (in minutes) spent eating between the: (i) GR 68755 and placebo group {mean 18.24 ± 1.2 Vs mean 18.20 ± 1.5; 95% CI -2.3 to 2.3, t = 0.04, df = 24, p=0.97} and those who had the (ii) GR 68755 with amphetamine and amphetamine combination {mean 14.5 ± 1.2 Vs 14.2 ± 1.6; 95% CI -4.0 to 3.1, t = -0.28, df = 23, p=0.78}.

On caloric intake, there was a statistically significant main effect of amphetamine. Subjects who received amphetamine alone consumed less calories than those who had placebo {estimate of treatment difference (estimate) -1846; 95% CI -2648 to -1045, df = 23, p<0.001}. Subjects who received GR 68755 and amphetamine had a smaller caloric intake compared to those who had amphetamine alone {estimate -1946; 95% CI -2736 to -1157, df = 23, p<0.001}. In contrast, there was no significant difference in caloric intake between: (i) the GR 68755 and placebo group {estimate 68; 95% CI -716 to 853, df = 24, p=0.86}, and (ii) the GR 68755 with amphetamine and the amphetamine alone group {estimate -32; 95% CI -834 to 770, df = 23, p=0.93}. The reduction in food intake by amphetamine affected both sweet and savoury items, and there was no sparing of any particular macronutrient {table XI and fig. XI}.

Computerized tests

On the RVIPT, amphetamine alone increased the number of correct responses per minute by 0.7 compared to placebo (mean 5.9 Vs 5.2; 95% CI 0.3 to 0.9; df = 66; $p<0.001$). GR 68755 also increased significantly the number of correct responses per minute by 0.3 per minute compared with placebo (mean 5.5 Vs 5.2; 95% CI 0 to 0.6; df = 66; p=0.03). GR 68775 with amphetamine increased the number of correct responses per minute by 0.5 compared with GR 68755 alone (mean 6.0 Vs 5.5; 95% CI 0.2 to 0.7; df = 66; p=0.003).

There was no statistically significant differences between the groups on geometric mean reaction time for both the RVIPT and SRT {see table XII}.

DISCUSSION

To minimize type I statistical errors, post-hoc estimates of treatment differences were only carried out if there was a main effect of treatment from the ANOVA or ANCOVA. Confidence intervals have also been included in the analysis, and differences were only taken to be biologically significant if they achieved the higher significance level of 1%.

While the ability of amphetamine to elevate mood and overall subjective state, induce reinforcement, and attenuate hunger were clearly demonstrated,

the ineffectiveness of the 5-HT_3 antagonist, GR 68755, on these changes was surprising and contrasts with the findings of the previous experiment. There can, however, be several reasons for this discrepancy. It is conceivable that serum levels, and possibly central nervous system penetration, of the 5-HT_3 antagonist was higher in the pilot study (experiment 4) which used a repeated dosing paradigm in contrast to the single dose method in the present experiment; however, this cannot be confirmed as serum levels were not measured directly. Single dosing of this second generation 5-HT_3 antagonist, GR 68755, was employed because: (i) it has a shorter half-life than ondansetron {for GR 68755 $t_{1/2}$ = 1.6 h Vs ondansetron 3 h); (ii) peak oral absorption occurs within 0.75-1 h of ingestion; (iii) it has been shown that 2 mg given intravenously can reverse the cognitive deficits following scopolamine in humans (Glaxo unpublished data on file).

It is, however, conceivable that the answer, though more complex, might be related to the precise mechanism by which 5-HT_3 antagonists oppose dopaminergic neurotransmission. It is noteworthy that a single oral dose of ondansetron was sufficient to attenuate the reinforcing effects of a small dose of alcohol in previous experiments. It may be, therefore, that 5-HT_3 antagonists are most effective at reducing reinforcement when the drug of abuse depends, like alcohol or morphine, on intact transmission at excitatory 5-HT_3 receptors, presumably located pre-synaptically on dopamine neurones. While this serotonin-dopamine interaction is quiescent under basal conditions, its activation by drugs like alcohol or morphine increases dopaminergic firing.

In contrast, while amphetamine and cocaine cause the direct release of dopamine from the synaptic cleft and prevent re-uptake, they do not increase dopaminergic transmission through this transynaptic mechanism. Thus, their reinforcing properties may not rely on intact 5-HT activity at pre-synaptic 5-HT_3 receptors. Nevertheless, this hypothesis can not account for the conflicting finding in animals that 5-HT_3 antagonists can block amphetamine-induced hyperlocomotion and *CPP* (Costall et al., 1989; Van der Hoek et al., 1989), and in some cases, fail to attenuate amphetamine (Carboni et al., 1989; Moser, 1992) and cocaine-induced behaviours (Paris and Cunningham 1991; Peltier and Schenk, 1991). In addition, it is possible that the effects of 5-HT_3 antagonists on drugs that release dopamine directly are not robust, or may be due to post-synaptic effects or interactions with other neurotransmitter systems which are at present poorly understood .

GR 68755 alone had no effect on any of the measures of mood or reinforcement, and this is in keeping with the findings of others (Costall et al., 1992). Amphetamine did not increase ratings of suspiciousness significantly, suggesting that these effects in psychotic patients may be attributed to chronic use, high dosages, or idiosyncratic reactions (Connell, 1958). The increase in restlessness when those who received GR 68755 and amphetamine were compared to the GR 68755 alone group contrasts with the results of animal studies which have suggested a putative role for 5-HT_3 antagonists as anxiolytics (Jones et al., 1988) but is in keeping with the results of a recent preliminary study in humans where the 5-HT_3 antagonist BRL

43694 failed to block the anxiogenic properties of the 5-HT agonist, m-Chlorophenylpiperazine (Silverstone, P; personal communication, 1993).

In the present experiment, I confirmed the findings of other investigators that amphetamine has anorectic properties in humans (Blundell and Rogers, 1980; Silverstone et al., 1980; Blundell, 1985). However, only one small study with 12 human subjects has examined the effects of *d*- and *l*- fenfluramine and amphetamine on macronutrient intake (Goodall et al., 1983). Like the present study which employed 26 subjects, amphetamine reduced the intake of all macronutrients. In contrast, *d*-fenfluramine selectively reduced the intake of carbohydrates but not fat or protein from all foods. In addition, when sweet and non-sweet foods were examined independently, *l*-fenfluramine reduced fat and carbohydrate intake from non-sweet foods but *d*-fenfluramine did not reduce carbohydrate intake from sweet foods. Neither *d*- nor *l*-fenfluramine reduced protein intake.

It is of interest that GR 68755 enhanced cognitive processing in normal human volunteers, by increasing the number of correct responses. This finding is in keeping with the suggestion from preliminary animal studies that 5-HT_3 antagonists reverse the deleterious effects of scopolamine on cognitive functioning in animals and humans (Domeney et al., 1991; Glaxo unpublished data on file) presumably by facilitation of cholinergic transmission. Further, in a pilot study in humans, ondansetron improved age related memory impairment (Crook and Larkin, 1991). While amphetamine also increased the number of correct responses on the RVIPT it did not improve reaction time on the computerized tests. This differential effect of amphetamine suggests it may improve sub-optimal performance due to boredom or fatigue, presumably via increased catecholamine release, but may have little effect on maximal performance (Weiss and Laties, 1962). Interestingly, the cognitive enhancing

properties of GR 68755 and amphetamine were not additive suggesting the response might already have been maximal.

14. GENERAL CONCLUSIONS

1. In humans, like animals, it appears that the reinforcing properties of alcohol may be related to its ability to facilitate dopaminergic transmission via excitatory activity at pre-synaptic 5-HT_3 receptors. The present thesis provides the first clear evidence in humans that the 5-HT_3 antagonist, ondansetron, which opposes dopaminergic transmission in the nucleus accumbens, can reduce the reinforcing value of alcohol. Importantly, it has also been demonstrated that the effects of ondansetron on alcohol consumption could not be explained by possible pharmacokinetic interactions. Notably, alcohol consumption was not measured directly and in this respect it is important to point out that there is evidence, from the study of other psychostimulants such as cocaine, that attenuation of its reinforcing effects might, paradoxically, increase consumption and the subject works harder to obtain the reinforcer. In future, I intend to investigate which dosages of ondansetron can cause a measurable decrease in alcohol consumption in normal human volunteers and alcohol abusers using the present human laboratory paradigm. If these studies are successful, this research will progress to clinical trials.

2. It remains unclear whether 5-HT_3 receptor antagonists attenuate the reinforcing and mood elevating effects of psychostimulants such as

amphetamine which cause the direct release and blockade of dopamine re-uptake from nerve terminals but do not increase dopamine neurotransmission transynaptically. Clarification of this issue would require a dose-response experiment, perhaps with repeated or parenteral administration of the 5-HT_3 antagonist. The potential cognitive enhancing properties of 5-HT_3 antagonists deserves further study, particularly in relation to the age, and possibly, disease-related memory impairment.

3. 5-HT_3 receptor antagonists on their own are without apparent effects on mood and do not induce reinforcing behaviour. This suggests their abuse liability is low, an issue which may be of critical importance if they are to fulfil their promise as being useful in the treatment of drug-seeking behaviour.

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Table I: Three-way repeated measures analysis of variance results for the change in mean score from baseline over time on visual analogue scales of subjective mood in sixteen healthy male volunteers receiving alcohol following pre-treatment with ondansetron.

	Alcohol x time	Ondansetron x time	Alcohol x ondansetron x time
	F; p	F; p	F; p
Visual analogue scales			
Cheerful	2.16; 0.04	2.72; 0.01	2.34; 0.03
Confident	1.19; 0.31	2.69; 0.13	3.70; 0.001
Energetic	2.10; 0.05	1.78; 0.09	2.92; 0.008
Feel good	2.97; 0.007	1.85; 0.09	1.29; 0.26
Optimistic	2.31; 0.03	1.73; 0.11	2.54; 0.02
Clear-headed	4.35; 0.0003	2.10; 0.05	0.95; 0.47
Agreeable	3.14; 0.005	3.32; 0.003	0.90; 0.51
Content	5.89; <0.0001	1.45; 0.19	2.91; 0.008
Relaxed	6.21; <0.0001	0.78; 0.60	1.08; 0.38
Desire for a drink	4.22; 0.0004	3.02; 0.006	6.92; <0.0001

For table, df=7, 105.

Table II: Effects of alcohol and ondansetron on change in visual analogue scale measures of subjective mood from baseline in sixteen healthy male volunteers.

Visual Analogue Scales of subjective mood in (mm)	Plac. Mean N=16	Ondan Mean N=16	Alc. Mean N=16	Alc. & Ondan Mean N=16	Estimate Alc. - Plac. {95% CI}	Estimate Ondan - Plac. {95% CI}	Estimate Alc. - Alc. & Ondan {95% CI}	Estimate Alc. & Ondan - Ondan {95% CI}
Energetic	3	3	7	1	3 {0, 7}; ns	0 {-3, 3}; ns	5 {2, 9}**	1 {-2, 4}; ns
Relaxed	4	2	18	7	14 {10, 17}	-2 {0, -4}	8 {5, 11}	8 {4, 10}
Feel good	8	1	14	5	7 {3, 10}	-7 {-4, 10}	10 {7, 12}	4 {0, 7}
Cheerful	5	5	10	2	5 {8, 2}*	0 {-3, 3}; ns	9 {6, 12}**	3 {0, 7}*
Agreeable	2	4	12	4	10 {13, 7}	2 {5, 0}	7 {5, 10}	0 {-3, 3}
Confident	5	4	11	3	5 {8, 2}*	-1 {-4, 8}; ns	8 {6, 11}**	-1 {-4, 2}; ns
Clear-headed	-2	-2	-6	-4	-4 {0, 7}	0 {-2, 2}	-2 {-5, 1}	-2 {-2, 5}

Table II contd.

Visual Analogue Scales of subjective mood in (mm)	Plac. Mean N=16	Ondan Mean N=16	Alc. Mean N=16	Alc. & Ondan Mean N=16	Estimate Alc. - Plac. {95% CI}	Estimate Ondan - Plac. {95% CI}	Estimate Alc. - Alc. & Ondan {95% CI}	Estimate Alc. & Ondan - Ondan {95% CI}
Desire for a drink	-10	-8	12	-8	21 {16, 26}**	-2 {-5, -2}; ns	20 {15, 25}**	0 {-4, 5}; ns
Content	2	1	11	8	9 {6, 12}**	0 {-2, 2}; ns	3 {0, 6}*	7 {9, 4}**
Optimistic	2	1	9	1	6 {4, 9}**	-1 {-9, 4}; ns	8 {5, 10}**	0 {-2, 2}; ns

For table df = 111. p>0.05=ns; p<0.0001=**.
Key: Plac. = Placebo; Alc. = Alcohol; Ondan = Ondansetron; CI = Confidence Interval; and estimate = estimate of treatment differences. Means and confidence intervals have been rounded up to the next whole number and may not add up. In the table the treatment groups have been summarized according to their main interactions: placebo and placebo (placebo); ondansetron and placebo alcohol (ondansetron); alcohol and placebo ondansetron (alcohol); and alcohol and ondansetron (alcohol and ondansetron).
To minimize type I errors, inter-group differences on the post-hoc paired t-tests were only carried out if the alcohol x ondansetron x time interactions on the preliminary three-way repeated measures ANOVA for that variable had achieved statistical significance at the 5%
level (see table I).

Table III: Comparison of the area under curve analyses for change in visual analogue scales (VAS) of mood for the periodbetween alcohol and dosing with: (a) ondansetron (ondansetron group) or (b) matching placebo (placebo group) in twelve healthy male volunteers.

	Placebo group		Ondansetron group			
	Median	{Interquartile range}	Median	{Interquartile range}		
	N=12		N=12		Z	p
VAS in mm						
Agreeable	-15	{143}	30	{113}	-0.47	0.63
Cheerful	60	{263}	30	{165}	-0.80	0.42
Clear-headed	30	{195}	0	{315}	-1.64	0.10
Confident	15	{202}	60	{173}	-1.18	0.23
Content	0	{225}	150	{232}	-0.35	0.72
Desire for a drink	-15	{330}	30	{375}	-1.51	0.13
Optimistic	-15	{188}	0	{345}	-0.56	0.57

For the VAS "buzz" the rating between alcohol and dosing with either ondansetron and placebo was 0 for both groups.

Interquartile ranges have been rounded up to the next whole number.

Table IV: Comparison of the area under curve analyses for change in visual analogue scales (VAS) for mood following alcohol in twelve healthy male subjects pre-treated with ondansetron or placebo at baseline.

	Alcohol and ondansetron group Median {Interquartile range} N=12		Alcohol and placebo group Median {Interquartile range} N=12		Z	p
VAS in mm						
Agreeable	-11	{223}	303	{509}	-1.96	0.049
Buzz	806	{764}	1118	{1243}	-0.78	0.43
Cheerful	38	{221}	93	{334}	-1.06	0.28
Clear-headed	-177	{345}	-149	{340}	-0.94	0.35
Confident	-24	{263}	305	{449}	-1.88	0.06
Content	23	{390}	187	{369}	-2.31	0.02
Desire for a drink	-388	{694}	58	{410}	-2.98	0.002
Optimistic	-40	{419}	274	{301}	-2.98	0.002

Medians and interquartile ranges have been rounded up to the next whole number.

Table V: Means and standard errors (s.e.m), and 95% confidence intervals (CI) for serumalcohol concentration following alcohol in eight healthy male volunteers pre-treated with ondansetron or placebo.

Time post alcohol (min)	Serum alcohol concentration following alcohol and ondansetron (mg/100 ml) Mean ± {s.e.m} N=8	Serum alcohol concentration following alcohol and placebo (mg/100 ml) Mean ± {s.e.m} N=8	95% CI for differences in mean serum alcohol concentration following alcohol and placebo - alcohol and ondansetron (mg/100 ml) Difference {95% CI}
3	1.1 ± {0.6}	1.4 ± {0.6}	-0.3 {-2.3, 1.6}
5	2.9 ± {1.5}	3.9 ± {2.2}	-1.1 {-3.9, 1.8}
10	6.4 ± {1.5}	8.0 ± {2.3}	-1.6 {-5.8, 2.6}
15	8.7 ± {1.6}	10.1 ± {2.3}	-1.4 {-7.5, 4.7}
20	15.3 ± {3.1}	15.6 ± {2.9}	-0.3 {-6.3, 5.7}
25	18.1 ± {2.6}	17.8 ± {3.1}	0.4 {-5.0, 5.8}
30	17.7 ± {2.2}	19.5 ± {3.1}	-1.8 {-6.2, 2.5}

Numbers have been rounded up to one decimal place and may not add up.

Table VI: Correlation between breath and serum alcohol concentrations following alcohol in eight healthy male volunteers pre-treated with ondansetron or placebo.

Time post alcohol (min)	Serum alcohol concentration following alcohol and ondansetron (mg/100 ml) Mean ± {s.e.m} N=8	Breath alcohol concentration following alcohol and ondansetron (mg/100 ml) Mean ± {s.e.m} N=8	Correlation between serum and breath alcohol concentration following alcohol and ondansetron r; p	Serum alcohol concentration following alcohol and placebo (mg/100 ml) Mean ± {s.e.m} N=8	Breath alcohol concentration following alcohol and placebo (mg/100 ml) Mean ± {s.e.m} N=8	Correlation between serum and breath alcohol concentration following alcohol and placebo r; p
20	15.3 ± {3.1}	23.8 ± {2.6}	0.4; 0.3	15.6 ± {2.9}	23.1 ± {4.0}	0.7; 0.03
25	18.1 ± {2.6}	27.5 ± {2.8}	0.6; 0.1	17.8 ± {3.1}	25.0 ± {4.8}	0.8; 0.2
30	17.7 ± {2.2}	30.0 ± {3.8}	0.6; 0.08	19.5 ± {3.1}	28.8 ± {4.8}	0.5; 0.2

Correlations were calculated using Pearson's correlation coefficient

Table VII: Effects of GR 68755 and *d*-amphetamine on subjective mood, hunger, and suspiciousness in healthy male volunteers.

Visual Analogue Scales (mm)	Plac.	GR 68755	*d*-amp	*d*-amp + GR 68755	Estimate *d*-amp - Plac.	Estimate GR 68775 - Plac.	Estimate GR 68775 & *d*-amp - *d*-amp	Estimate GR 68775 & *d*-amp - GR 68775
	Mean	Mean	Mean	Mean	{95% CI}	{95% CI}	{95% CI}	{95% CI}
Mood/arousal								
Confident	45	45	50	50	4 {2, 7}**	0 {-2, 2}; ns	0 {-2, 2}; ns	4 {2, 7}**
Cheerful	45	45	50	50	4 {2, 7}**	0 {-3, 2}; ns	0 {-2, 3]; ns	5 {3, 7}**
Mind racing	1	1	8	8	7 {3, 12}**	0 {-4, 5}; ns	0 {-4, 5}; ns	7 {3, 11}**
Alertness	44	44	53	54	9 {5, 13}**	0 {-3, 4}; ns	1 {-3, 5}; ns	9 {5, 13}**
Restless	6	6	9	11	2 {-1, 6}; ns	0 {-3, 3}; ns	2 {-1, 5}; ns	5 {2, 8}*
Lethargic	44	42	36	35	-8 {-12, -4}**	-1 {-5, 2}; ns	0 {-4, 4}; ns	-7 {-11, -3}**
Reinforcement								
Feeling good	3	4	12	12	9 {5, 14}**	1 {-4, 5}; ns	0 {-5, 4}; ns	8 {4, 13}**
Buzz	0	1	7	8	7 {3, 11}**	0 {-4, 4}; ns	1 {-3, 5}; ns	7 {4, 11}**

Table VII contd. Visual Analogue Scales (mm)	Plac.	GR 68755	*d*-amp	*d*-amp + GR 68755	Estimate *d*-amp - Plac.	Estimate GR 68775 - Plac.	Estimate GR 68775 & *d*-amp - *d*-amp	Estimate GR 68775 & *d*-amp - GR 68775
	Mean	Mean	Mean	Mean	{95% CI}	{95% CI}	{95% CI}	{95% CI}
Anorectic ability								
Hunger	46	48	37	40	-9 {-13, -5}**	1 {-2, 5}; ns	3 {-1, 7}, ns	-8 {-12, -4}**
Strong wish to eat	41	42	30	32	-11 {-15, 6}**	1 {-3, 6}; ns	1 {-3, 6}; ns	-10 {-15, -6}**
Suspiciousness								
Feeling bothered	18	17	18	18	0 {-1, 2}; ns	-1 {-3, 1}; ns	0 {-2, 2}; ns	1 {-1, 3}; ns
Treated fairly	64	64	65	66	1 {-1, 2}; ns	0 {-1, 2}; ns	1 {0, 3}; ns	1 {0, 3}; ns

For table df = 66 for estimates of treatment differences. p>0.05=ns; p<0.001=**.
Key: Plac. = Placebo; *d*-amp = *d*-amphetamine; CI = Confidence Interval; and estimate = estimate of treatment differences.
To minimize type I errors, inter-group differences on the post-hoc t-test were only considered to be significant if the preliminary three-way repeated measures ANOVA for that variable achieved statistical significance at the 5% level.

Table VIII: Analyses of covariance for visual analogue scales (VAS) measuring mood/arousal, reinforcement, anorectic ability, and suspiciousness.

VAS (mm)	Baseline df; F; p - value	Period df; F; p - value	Treatment df; F; p - value
Mood/arousal			
Confident	1; 0.38; 0.53	3; 3.4; 0.01	3; 9.77; <0.001
Cheerful	1; 1.5; 0.21	3; 1.3; 0.27	3; 10.20; <0.001
Mind racing	1; 3.9; 0.06	3; 1.7; 0.16	3; 7.65; <0.001
Alertness	1; 5.9; 0.01	3; 0.41; 0.74	3; 14.72; <0.001
Restless	1; 0.002; 0.96	3; 3.03; 0.04	3; 4.23; 0.008
Lethargic	1; 10.8; 0.002	3; 0.11; 0.95	3; 10.20; <0.001
Reinforcement			
Feeling good	1; 0.65; 0.42	3; 2.04; 0.15	3; 9.86; <0.001
Buzz	1; 0.92; 0.33	3; 2.43; 0.07	3; 8.79; <0.001
Anorectic ability			
Hunger	1; 17.09; <0.001	3; 1.05; 0.37	3; 14.48; <0.001
Strong wish to eat	1; 12.56; <0.001	3; 1.84; 0.83	3; 14.05; <0.001
Suspiciousness			
Feeling bothered	1; 154.71; <0.001	3; 0.17; 0.91	3; 0.54; 0.65
Treated fairly	1; 142.15; <0.001	3; 0.20; 0.89	3; 2.01; 0.12

For table, df = 3, 66. Subjects = 24.

Table IX: Effects of GR 68755 and *d*-amphetamine on visual analogue scales (VAS) for satiety.

VAS (mm)	Plac.	GR 68755	*d*-amp	*d*-amp + GR 68755	Paired t-test	Paired t-test	Paired t-test	Paired t-test
					Plac. x *d*-amp	GR 68755 & *d*-amp x Plac.	GR 68755 & *d*-amp x *d*-amp	GR 68755 x Plac.
	Mean ± s.e.m	Mean ± s.e.m	Mean ± s.e.m	Mean ± s.e.m				
		[F; p]	[F; p]	[F; p]	t	t	t	t
	N=24	N=25	N=24	N=24	[p-value]	[p-value]	[p-value]	[p-value]
Pre-meal								
I expect to find the meal satisfying	63 ± 3	61 ± 2	48 ± 3	46 ± 3	3.1	5.5	0.35	1.7
		[0.15; 0.69]	[37.4; <0.0001]	[0.31; 0.57]	[0.004]	[<0.0001]	[0.72]	[0.11]
Right now I could eat a large amount of food	62 ± 3	57 ± 3	37 ± 3	34 ± 4	4.4	5.2	0.36	1.6
		[1.13; 0.28]	[54.8; <0.0001]	[0.35; 0.55]	[<0.0001]	[<0.0001]	[0.72]	[0.12]

Table IX
contd.

VAS (mm)	Plac.	GR 68755	d-amp	d-amp + GR 68755	Paired t-test	Paired t-test	Paired t-test	Paired t-test
					Plac. x d-amp	GR 68755 & d-amp x Plac.	GR 68755 & d-amp x d-amp	GR 68755 x Plac.
	Mean ± s.e.m	Mean ± s.e.m	Mean ± s.e.m	Mean ± s.e.m				
		[F; p]	[F; p]	[F; p]	t	t	t	t
	N=24	N=25	N=24	N=24	[p-value]	[p-value]	[p-value]	[p-value]
Post-meal								
I found the meal satisfying	70 ± 2	66 ± 3	53 ± 4	53 ± 4	3.1	1.8	0.41	1.1
		[0.06; 0.81]	[22.3; < 0.0001]	[0.52; 0.47]	[0.004]	[0.09]	[0.68]	[0.27]
I feel full	77 ± 3	78 ± 3	68 ± 3	72 ± 2	2.7	3.7	-0.78	-0.03
		[0.99; 0.32]	[10.0; 0.002]	[0.47; 0.49]	[0.01]	[0.001]	[0.44]	[0.97]

Two-way repeated measures ANOVAs for main effects of GR 68755, *d*-amphetamine, and GR 68755 x *d*-amphetamine are given in their respective columns; for these, the df = 3, 66. For t-tests of all groups except GR 68755 x Placebo where df = 24, df = 23.
Key: **Plac. = placebo; *d*-amp = *d*-amphetamine.**

Table X: Responses on *'Forced'* and *'free'* food choice questionnaires before and after a test meal in human males pre-treated with placebo, GR 68755, *d*-amphetamine, and *d*-amphetamine and GR 68755.

Food choice conditions	Placebo	GR 68755	*d*-amphetamine	*d*-amphetamine and GR 68755
	Mean ± s.e.m	Mean ± s.e.m	Mean ± s.e.m	Mean ± s.e.m
	N=24	N=25	N=24	N=24
Pre-meal				
Forced choice protein	14 ± 1.4	13 ± 1.4	17 ± 1.4	17 ± 1.1
Free choice protein	3 ± 0.5	3 ± 0.4	5 ± 0.4	5 ± 0.4
Fat	3 ± 0.5	4 ± 0.4	5 ± 0.4	5 ± 0.4
Carbohydrate	2 ± 0.3	3 ± 0.4	4 ± 0.4	4 ± 0.4
Low energy	3 ± 0.5	3 ± 0.5	4 ± 0.5	3 ± 0.5

Table XI: Effect of GR 68755 and *d*-amphetamine on caloric intake and macronutrient selection in healthy males

Conditions (Calories)	Placebo	GR 68755	*d*-amphetamine	*d*-amphetamine + GR 68755
	Mean ± s.e.m	Mean ± s.e.m	Mean ± s.e.m	Mean ± s.e.m
Sweet foods				
Protein	208 ± 26	196 ± 30	145 ± 27	149 ± 25
Fat	491 ± 70	488 ± 71	312 ± 64	343 ± 64
Carbohydrate	1299 ± 134	1320 ± 144	986 ± 139	1030 ± 112
Savoury foods				
Protein	776 ± 55	813 ± 70	524 ± 59	519 ± 69
Fat	1800 ± 124	1827 ± 132	1209 ± 174	1103 ± 158
Carbohydrate	1100 ± 86	1065 ± 82	737 ± 100	754 ± 118
Total				
Protein	984 ± 61	1009 ± 77	668 ± 74	668 ± 75
Fat	2332 ± 132	2365 ± 161	1519 ± 197	1446 ± 192
Carbohydrate	2400 ± 176	2360 ± 176	1722 ± 195	3845 ± 423
Sum Total	5671 ± 307	5733 ± 346	3837 ± 432	3845 ± 423

Table XI
contd.

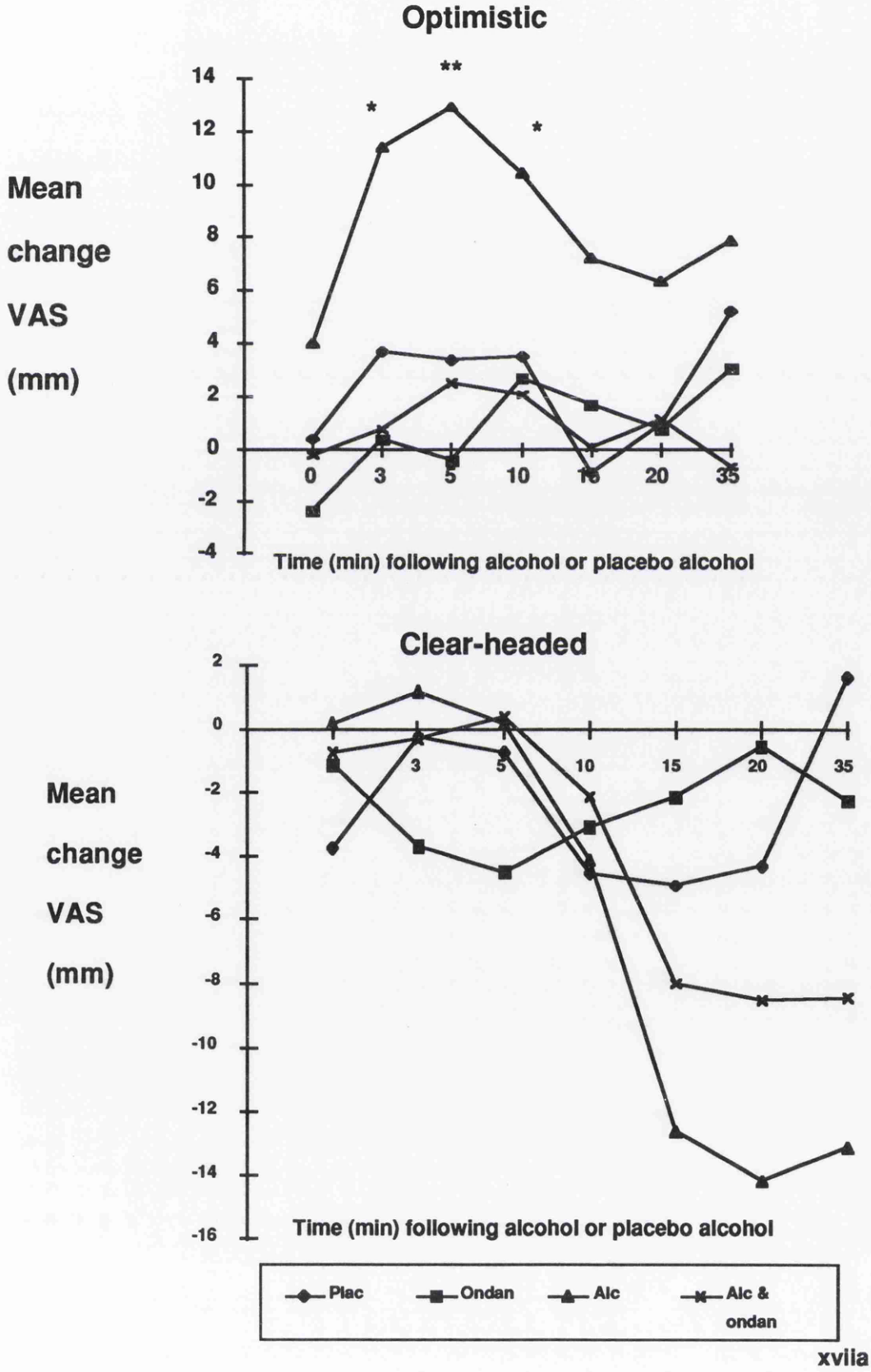
Food choice conditions	Placebo	GR 68755	*d*-amphetamine	*d*-amphetamine and GR 68755
	Mean ± s.e.m	Mean ± s.e.m	Mean ± s.e.m	Mean ± s.e.m
	N=24	N=25	N=24	N=24
Post meal				
Forced choice protein	7 ± 1.2	6 ± 1.2	7 ± 1.2	7 ± 1.3
Free choice protein	0 ± 0.1	0 ± 0.1	1 ± 0.1	0 ± 0.1
Fat	1 ± 0.4	1 ± 0.4	2 ± 0.3	2 ± 0.3
Carbohydrate	0 ± 0.4	1 ± 0.3	2 ± 0.3	2 ± 0.3
Low energy	2 ± 0.4	2 ± 0.3	3 ± 0.4	2 ± 0.4

Fig. XII: Effects of GR 68755 and d-amphetamine (d-amp) on computerized tests of cognitive performance in healthy male volunteers.

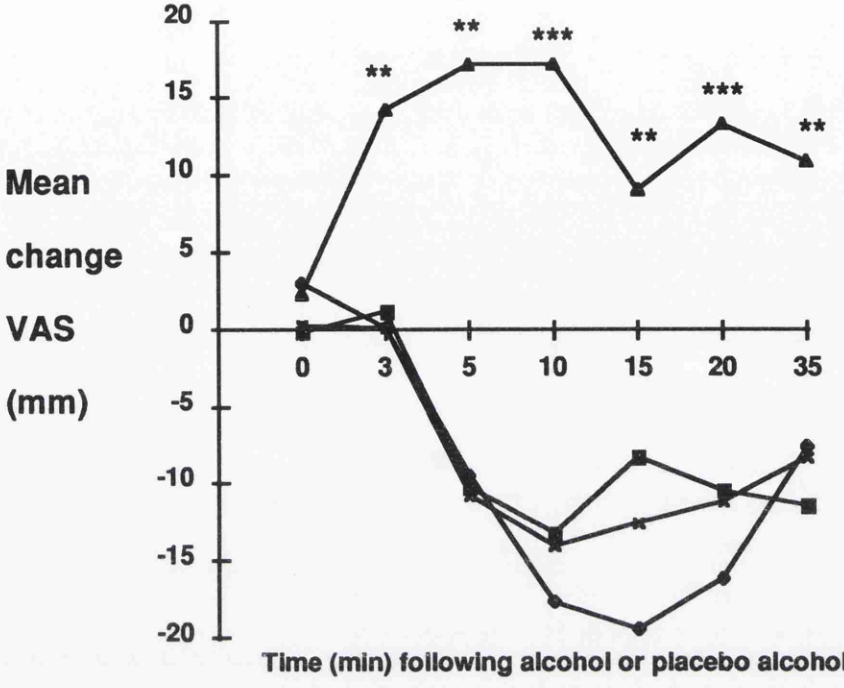
	Placebo Geometric Mean N=24	GR 68755 Geometric Mean N=25	*d*-amp Geometric Mean N=24	*d*-amp + GR 68755 Geometric Mean	Estimate *d*-amp - Plac. {95% CI} [p value]	Estimate GR 68775 - Plac. {95% CI} [p value]	Estimate GR 68775 & *d*-amp - *d*-amp {95% CI} [p value]	Estimate GR 68775 & *d*-amp - GR 68775 {95% CI} [p value]
Time taken to perform computerized Performance tests (milliseconds)								
Rapid visual information processing task								
Baseline	**508**	**500**	**493**	**506**				
3 h post d-amphetamine	**490**	**484**	**477**	**474**	**0.97** **{0.94, 1.00}** **[0.09]**	**0.99** **{0.96, 1.02}** **[0.41]**	**0.99** **{0.96, 1.02}** **[0.66]**	**0.98** **{0.95, 1.02}** **[0.17]**
Simple reaction time								
Baseline	**323**	**323**	**315**	**310**				
3 h post d-amphetamine	**322**	**320**	**317**	**310**	**0.98** **{0.95, 1.03}** **[0.44]**	**0.99** **{0.95, 1.03}** **[0.66]**	**0.98** **{0.94, 1.02}** **[0.26]**	**0.97** **{0.93, 1.01}** **[0.14]**

The geometric means post d-amphetamine are adjusted means derived from the least square means adjusted for baseline values. Numbers have been rounded up and may not add up. *Key*: Plac. = placebo; estimate = estimate of treatment differences.

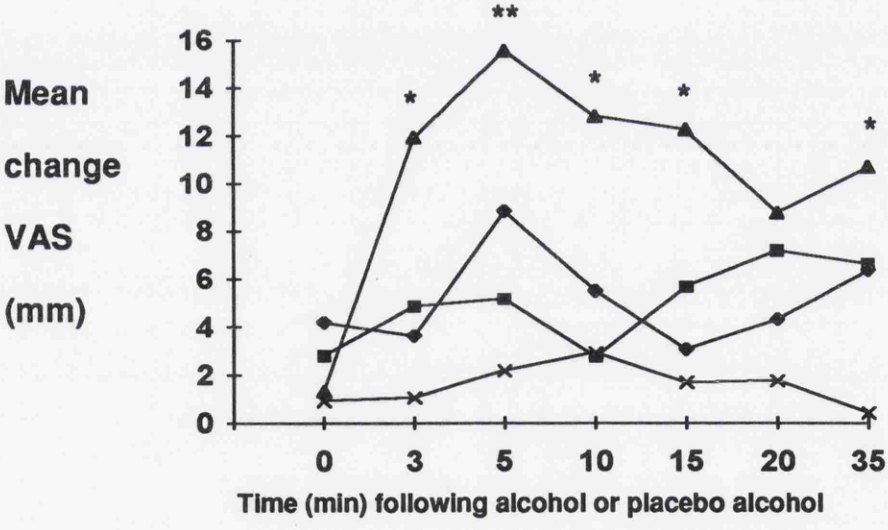
Fig.I. Mean change (mm) in visual analogue scales (VAS) for 16 subjects who received four treatment conditions: (a) alcohol and placebo ondansetron {alc}; placebo and placebo {plac}; placebo alcohol and ondansetron {ondan}, and (d) alcohol and ondansetron {alc & ondan}. Alcohol (3.6% alcohol content by volume of lager and 20 ml lime juice) or placebo alcohol was given at time "0", 60 min following ondansetron or placebo ondansetron. Asteriks show significant differences between alcohol and placebo and alcohol and ondansetron, *p<0.05, **p<0.02, ***p<0.001 (post hoc one way analysis of variance)



Desire for a drink



Cheerful



Confident

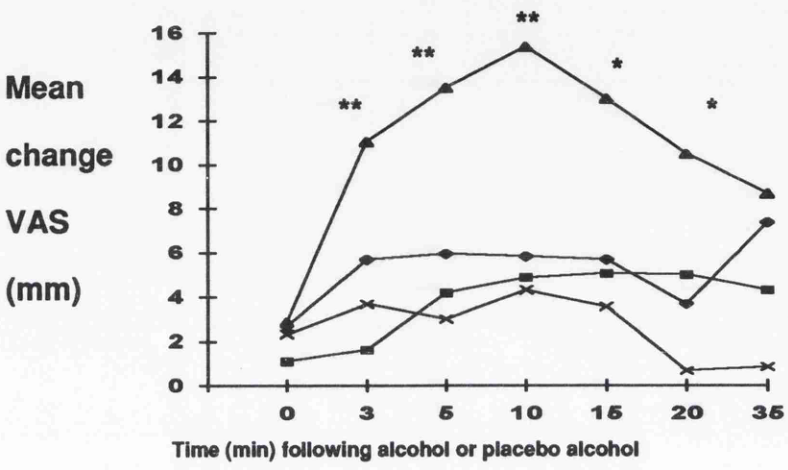
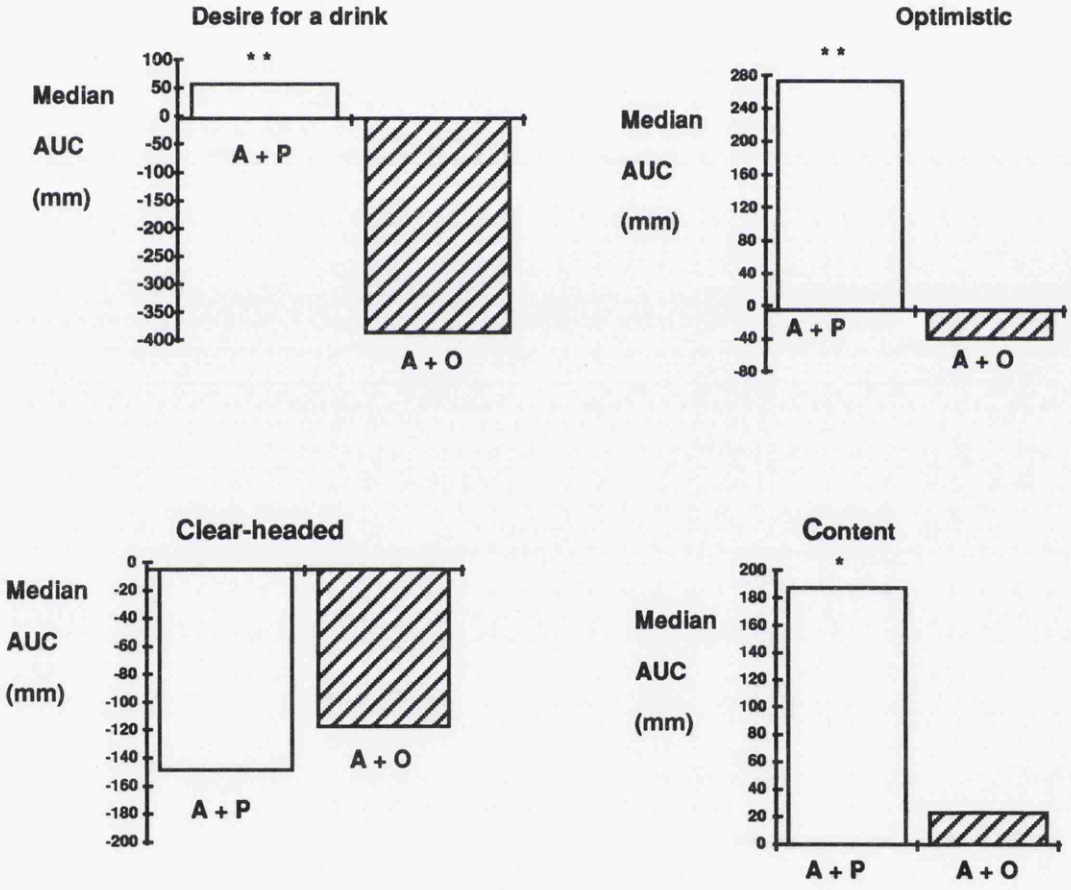
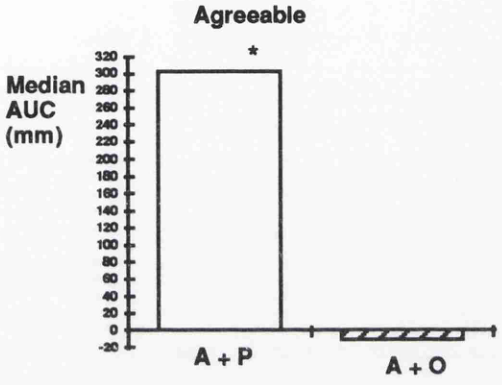
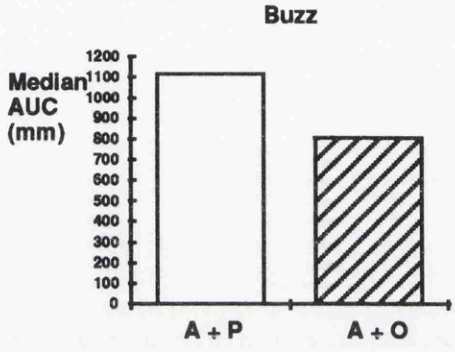
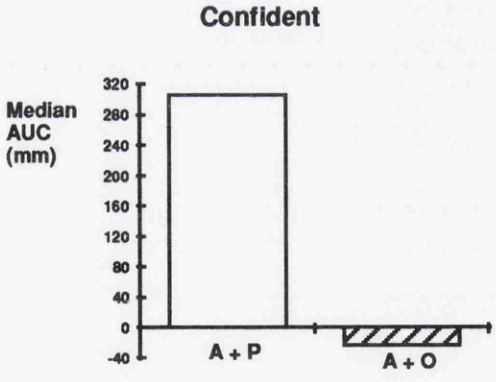


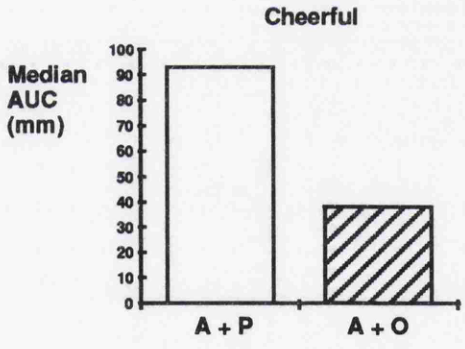
Fig. II. Medians of the area under curve analyses (AUC) for change in self-ratings of subjective mood following alcohol in 12 healthy males pre-treated with ondansetron or matching placebo











***Key*: A+ O = alcohol and ondansetron**

A + P = alcohol and placebo

p<0.05=*; P<0.01=**

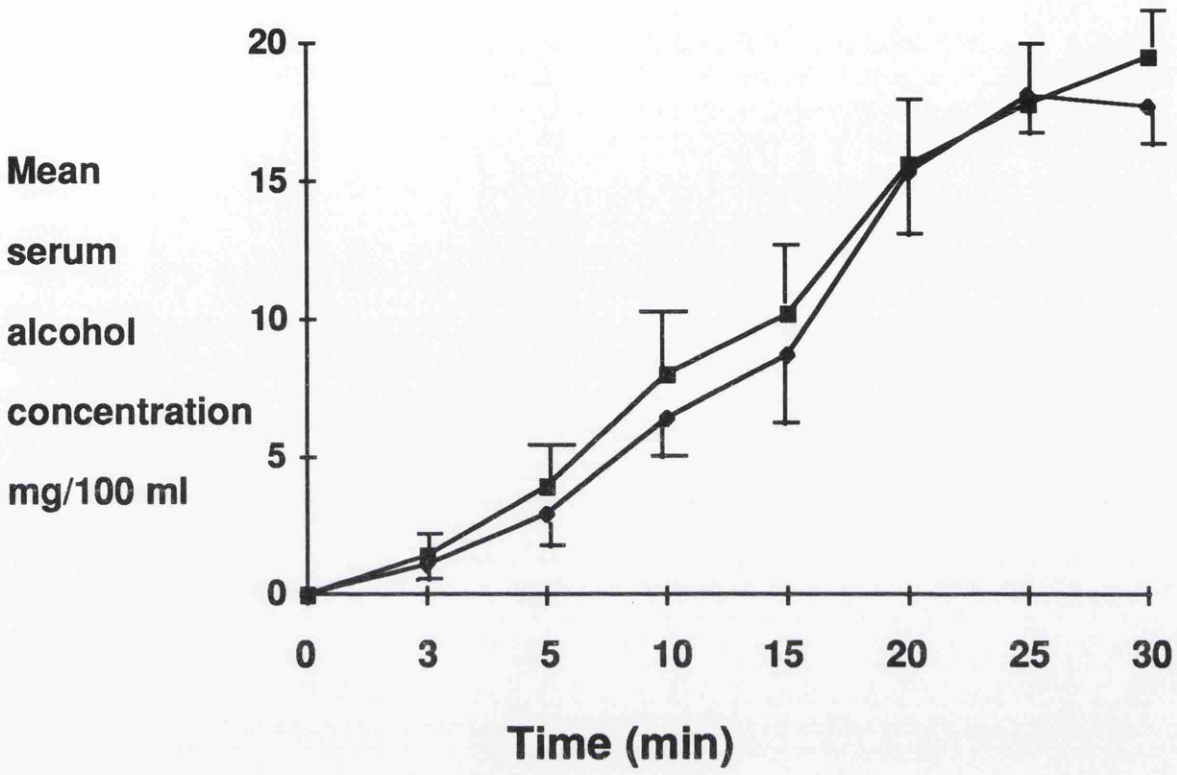


Fig. III. Mean serum alcohol concentration in 8 healthy males who received 580 ml of 3.6% alcohol content by volume of lager + 20 ml of lime juice following treatment with ondansetron or matching placebo

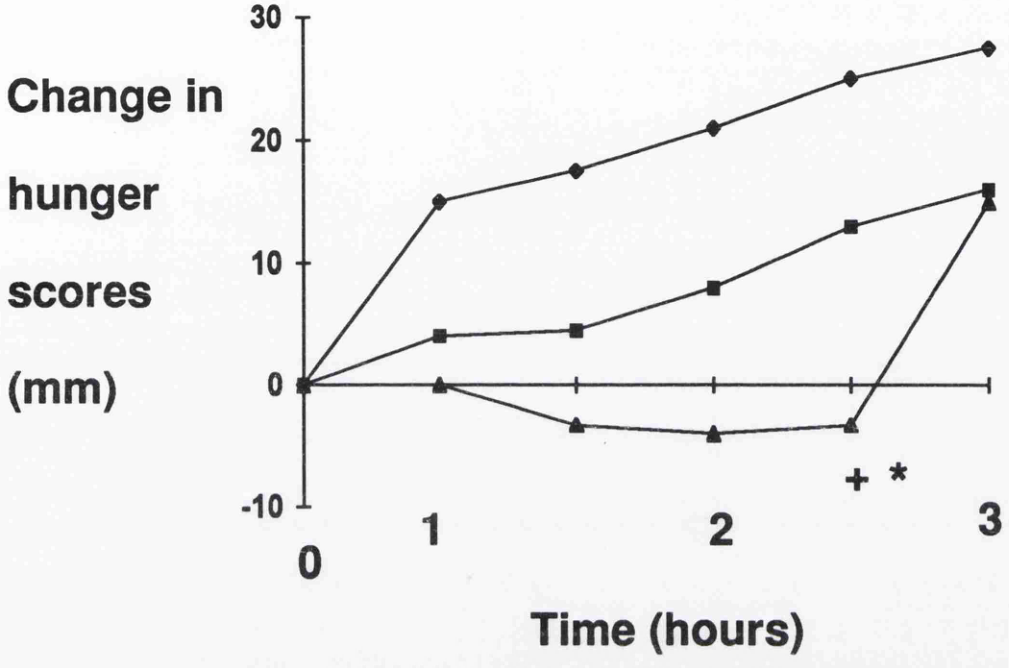


Fig. IV. Mean change from baseline (in mm) for self-ratings of hunger in 9 healthy males following either placebo and placebo, ondansetron and amphetamine, or placebo and amphetamine. * Significantly less than placebo and placebo, $p<0.05$, paired t-test. + Significantly less than ondansetron and amphetamine, $p<0.05$, paired t-test

Placebo/placebo

Ondansetron/amphetamine

Placebo/amphetamine

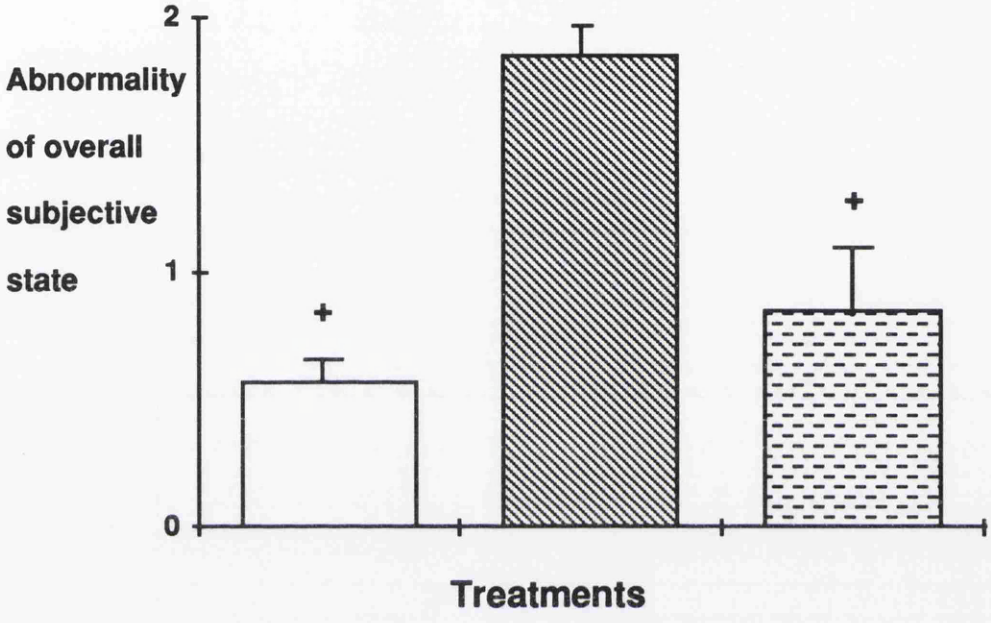


Fig. V. Overall subjective state in 9 healthy males who received placebo/placebo, placebo/amphetamine, or ondansetron/amphetamine. Both ondansetron/amphetamine and placebo/placebo scores were significantly different from the placebo/amphetamine group {+p<0.05, Wilcoxon's signed rank test}.

- Placebo/placebo
- Placebo/amphetamine
- Ondansetron/amphetamine

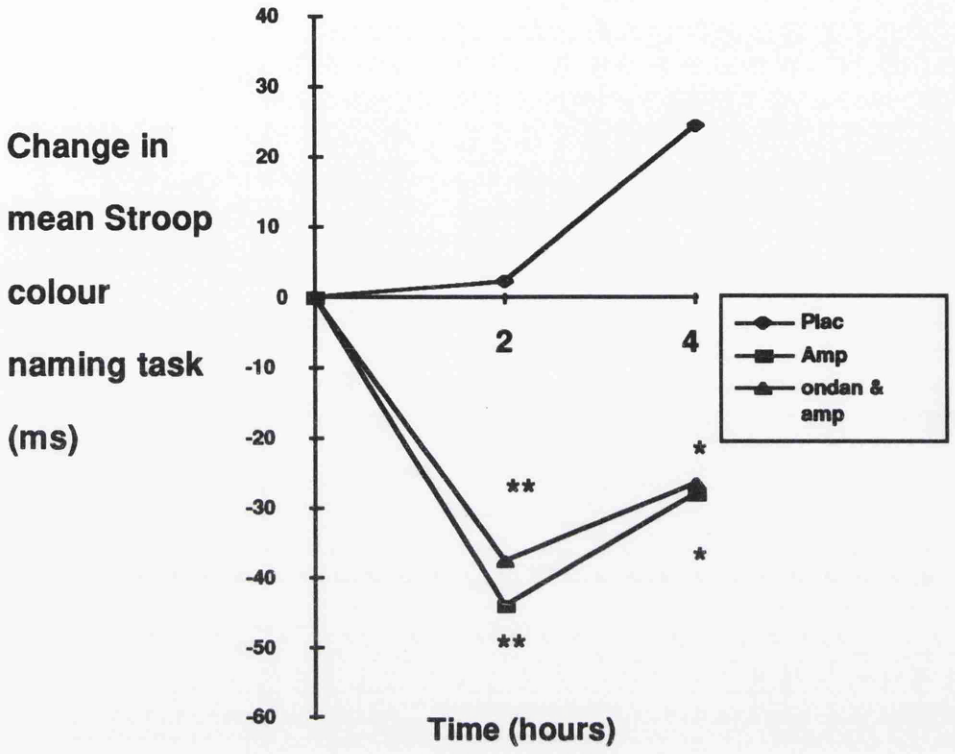


Fig. VI. Change in time taken to answer the Stroop colour naming task in 9 healthy males who received placebo and placebo {plac}, placebo and amphetamine (amp}, or ondansetron and amphetamine {ondan & amp}. For subjects who received placebo and amphetamine or ondansetron and amphetamine, significant differences from the placebo and placebo group are shown as *p<0.05 and **p<0.02; paired t-test

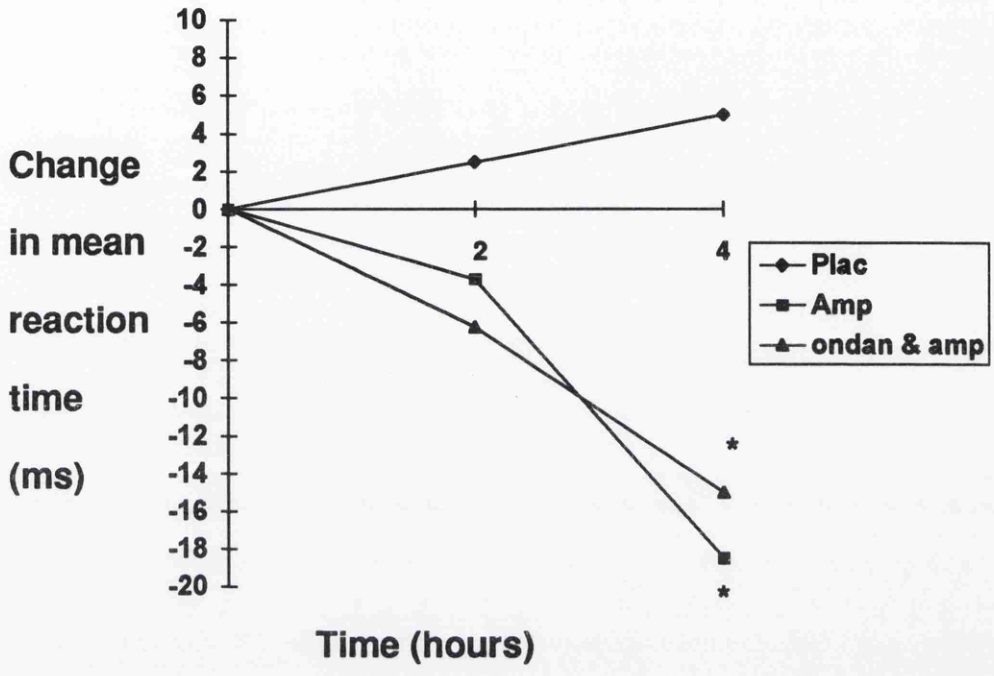


Fig. VII. Change in mean reaction time in 9 healthy males who received placebo and placebo {plac}, placebo and amphetamine {amp}, or ondasetron and amphetamine {ondan & amp}. For subjects who received placebo and amphetamine or ondansetron and amphetamine, significant differences from the placebo and placebo group were shown as *p<0.05; paired t-test

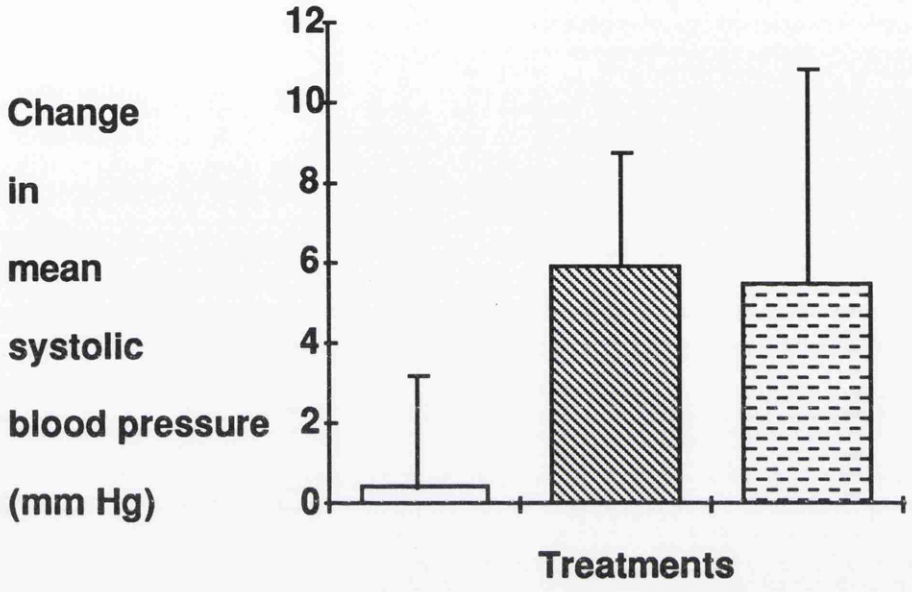


Fig. Viii. Change in mean systolic blood pressure in 9 healthy male volunteers who received placebo/placebo, ondansetron/amphetamine, or placebo/amphetamine. For both placebo/amphetamine and ondansetron/ amphetamine, significant differences from the placebo/ placebo group are shown as *p<0.05, paired t-test

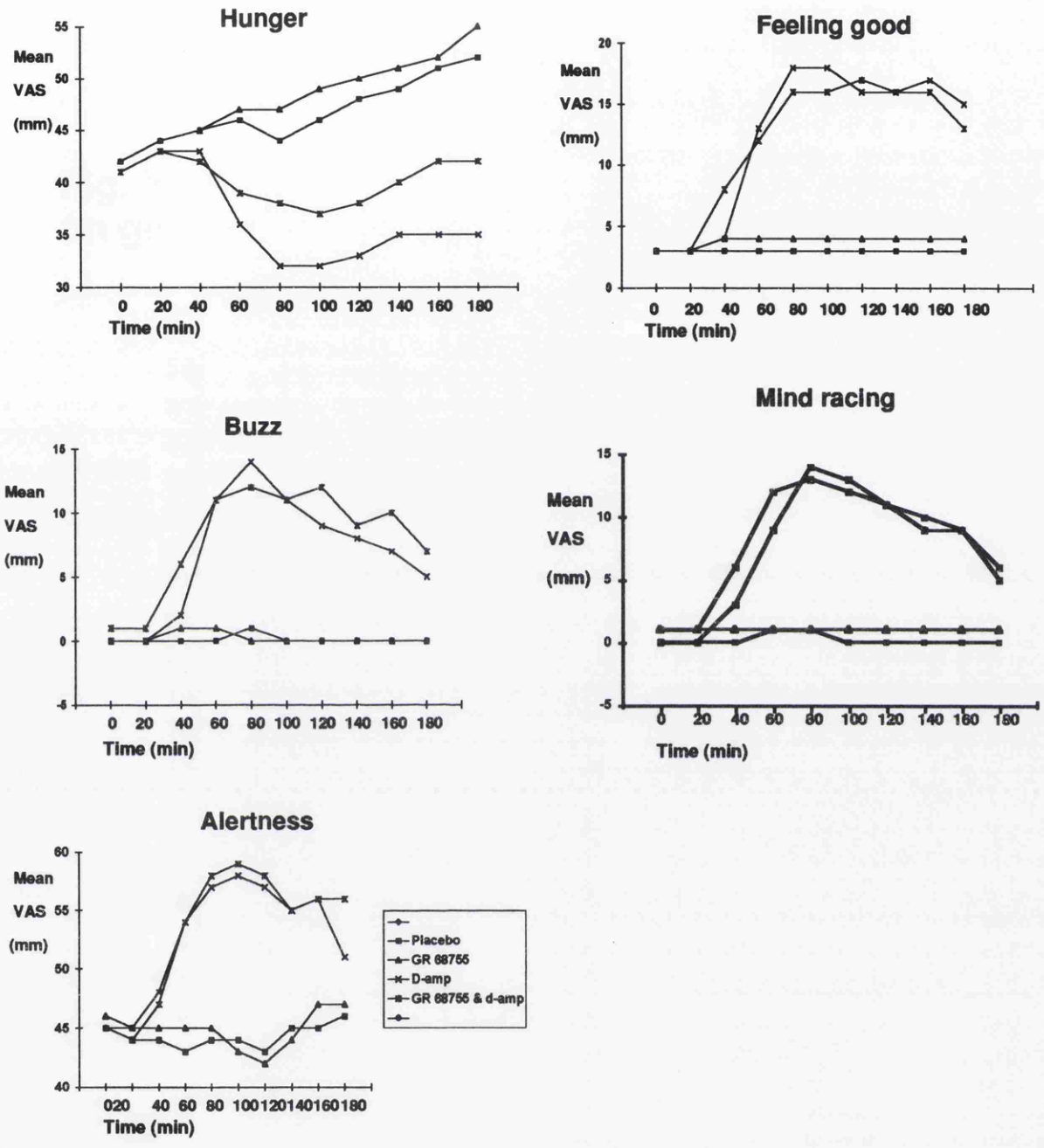
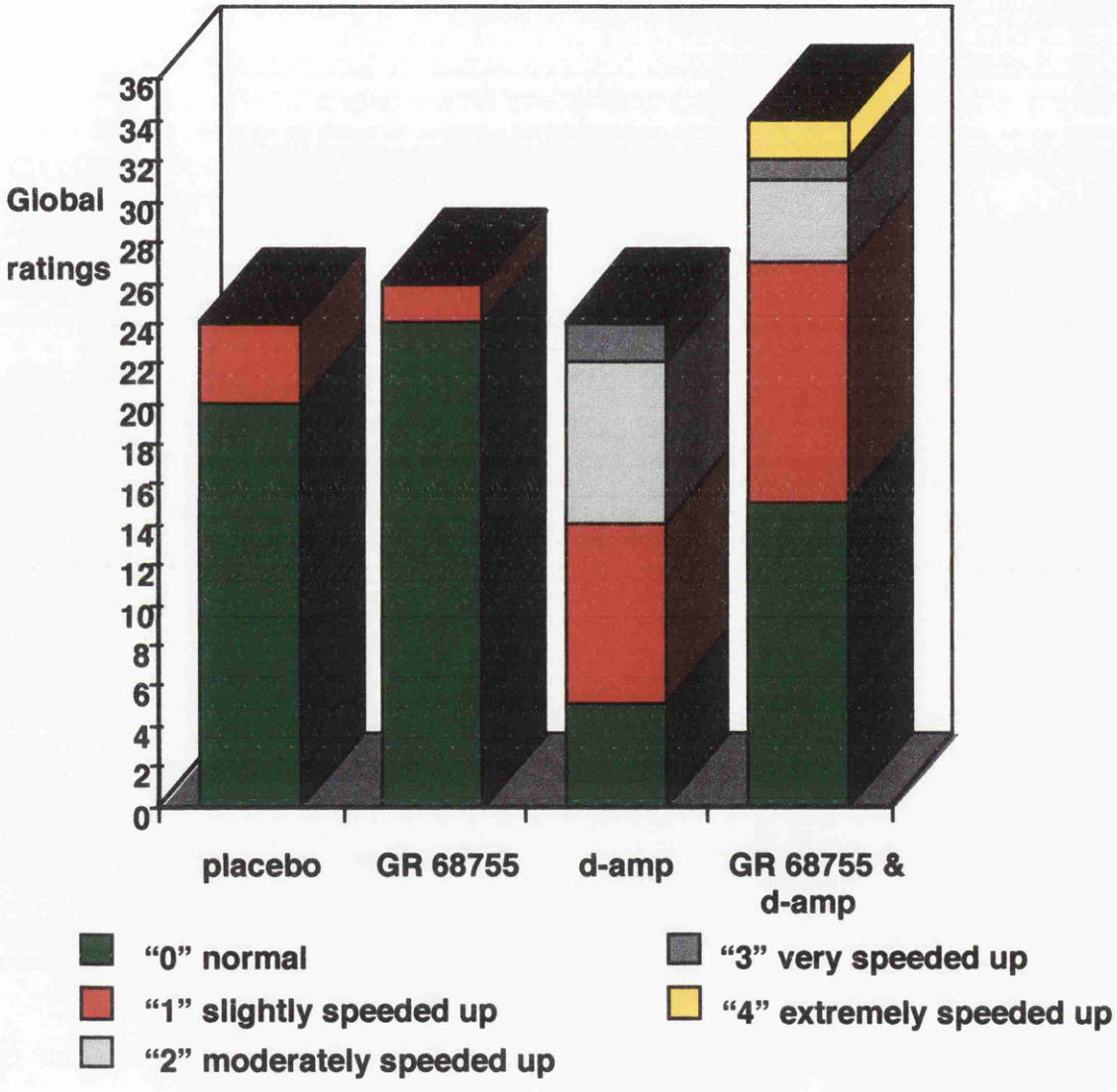


Fig. IX. Weighted means (mm) on visual analogue scales (VAS) for 24 subjects who received: (a) placebo + placebo {placebo}; (b) GR 68755 + placebo d-amphetamine {GR 68755}; (c) d-amphetamine (d-amp) + placebo {D-amp}; and (d) GR 68755 & d-amp {GR 68755 & d-amp}. GR 68755 (2 mg orally) was administered 30 min before d-amp. For graphs, d-amphetamine (20 mg orally) was administered at time "0", and there was a significant effect of d-amphetamine compared with placebo.

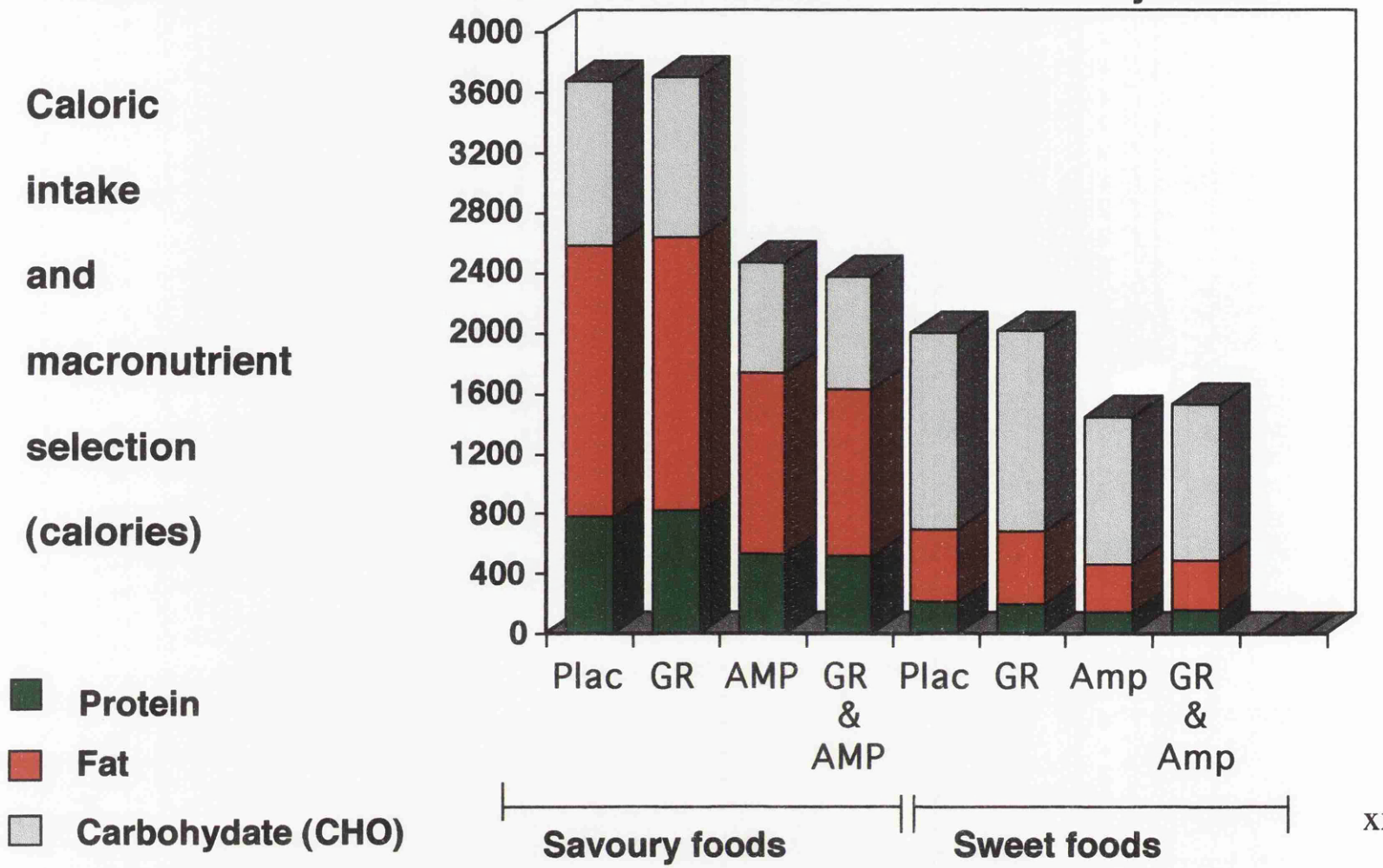
$p<0.001$ {paired t-tests}.

Fig. X: Effects of GR 68755 and d-amphetamine (d-amp) on global ratings of subjective mood in healthy males



Note: subject 14 received GR 68755 in two periods, with a score of 1 in period 1 and 0 in period 4.

Fig. XI: Effects of GR 68755 (GR) and d-amphetamine (Amp) on caloric intake and macronutrient selection in 24 healthy males



Appendix 1a: Sample visual analogue scales on mood and the desire to drink for experiment 1

Visual Analogue Scales

Subject ID..... Session ..... Date ..... Time .....

I feel confident

.....

Not at all **Extremely**

I feel cheerful

.....

Not at all **Extremely**

I feel energetic

.....

Not at all **Extremely**

I feel relaxed

.....

Not at all **Extremely**

I feel agreeable

.....

Not at all **Extremely**

I feel optimistic

.....

Not at all **Extremely**

I feel clear-headed

.....

Not at all **Extremely**

I feel optimistic

.....

Not at all **Extremely**

I feel like a drink

.....

Not at all **Extremely**

Appendix 1b details the volunteer screening procedure, inclusion, exclusion and withdrawal criteria, and study day restrictions for experiment 5.

Volunteer screening

- A medical and history and examination, and a psychiatric interview was performed in the two week period prior to each subject's first dosing occasion.
- Blood pressure (BP) was measured at the screening visit using an automated BP monitor. Subjects rested supine for 15 minutes after which three readings were taken separated by 5 minute intervals. Subjects were excluded if the mean systolic BP was greater than 140 mm Hg or the mean diastolic larger than 90 mm Hg.
- Laboratory safety tests (haematology, biochemistry and urinalysis) were performed at the screening visit, and a urine sample obtained for drugs of abuse including alcohol.
- In the one week period prior to the first dosing occasion, subjects were required to chose what they would like to eat during the test meal from a list of food items (see appendix 1d).

inclusion criteria

- Male volunteers aged between 18 and 45 years who are in good physical health and who have no history of mental illness or substance abuse.

Exclusion criteria

- Participation in a study within the previous four months.
- Being in receipt of a regular course of medication four weeks prior to the study.
- Consumption of more than 4 units of alcohol per day (1 unit =1/2 pint of normal strength beer, one glass of wine, or a single measure of spirit.
- Consumption of more than 8 cups of caffeinated coffee per day.
- Smoking more than 8 cigarettes per day (or the tobacco equivalent per day.
- Body weight greater than 100 kg or less than 60 kg.
- Vegetarians.

Withdrawal criteria

- Unwanted effects from the study drugs.
- Inter-current illness requiring medication.
- Abnormal laboratory tests judged to be of clinical importance.
- Subjects not wishing to continue with the study for any reason.

Study day restrictions

- No caffeinated beverages or smoking 12 hours preceding dose or during the study
- No alcohol for the 24 hour period preceding dose or during the study period.
- No strenuous exercise on study days.
- No concurrent medication on study days.
- Volunteers should not drive, cycle, or operate machinery for 24 hours following dosing.

Appendix 1c: Global rating scale for experiment 5.

Volunteer Number: ----------

Occasion Number: ----------

Date: ----------

Global rating Scale

Please circle your response

HOW DID THE TABLETS/CAPSULES MAKE YOU FEEL

0 - NORMAL

1 - SLIGHTLY LIGHT-HEADED, RESTLESS, OR SPEEDED UP

2 - MODERATELY LIGHT-HEADED, RESTLESS, OR SPEEDED UP

3 - VERY LIGHT-HEADED, RESTLESS, OR SPEEDED UP

4 - LIGHT-HEADED, RESTLESS, OR SPEEDED UP

Please enter any other comments you may have below:

.....

.....

.....

Appendix 1d: Food items from which subjects select the composition of their test meal

Volunteer Number: ..... Date: .....

Please rate the following foods according to your preference below:

1 2 3 4 5 6 7 8 9 10

Dislike Neutral Dislike

Extremely Extremely

Bread:

------White

------Soft Grain (Mighty White)

------Wholemeal

Sandwich Fillings and cold meats:

------Cheddar Cheese

------Cottage Cheese

------Peanut Butter

------Ham

------Tuna Fish

------Chicken Roll

------Marmite

------Strawberry Jam

Chocolate:

------Milk Chocolate (Cadbury's)

------Plain Chocolate (Bournville)

------White Chocolate (Milky Bar)

Biscuits:

-------Rich Tea

-------Morning Coffee

-------Digestive

Deserts:

-------Strawberry Yoghurt

-------Raspberry Yoghurt

-------Black Cherry Yoghurt

Crisps:

-------Ready Salted

-------Smoky bacon

-------Cheese and onion

Fruit:

-------Apple

-------Orange

-------Banana

xxx

Appendix 1e: The '*forced choice*' food questionnaire

Volunteer Number: ---------- Occasion Number: ----------

Date: ----------

Please examine each of the following pairs of food items one at a time and place a cross on the line next to one which you would prefer to eat at this moment. You must indicate your preference for one food item out of each pair.

A roast chicken	----or----	A baked potato with butter
1/4 lb. grilled Rump steak	----or----	A large Cadbury's Flake
A doughnut	----or----	A medium sized grilled cod fillet
2 oz of cheddar cheese	----or----	A diary king cone of ice cream
A dish of tinned fruit salad	----or----	Two poached eggs
A baked potato with butter	----or----	1/4 lb. grilled Rump steak
2 oz Cheddar cheese	----or----	A doughnut
A dairy king cone of ice cream	----or----	A roast chicken breast
A baked potato with butter	----or----	A medium sized grilled cod fillet
Two poached eggs	----or----	A large cadbury's flake
1/4 lb. grilled Rump steak	----or----	A doughnut
A dish of tinned fruit salad	----or----	2oz of Cheddar cheese
A roast chicken breast	----or----	A large Cadbury's flake
A baked potato with butter	----or----	Two poached eggs
A medium sized grilled cod fillet	--or----	A diary king cone ice cream
1/4 lb. grilled rump steak	----or----	A dish of tinned fruit salad
A large cadbury's flake	----or----	A medium sized grilled cod fillet
A roast chicken breast	----or----	A dish of tinned fruit salad
A doughnut	----or----	Two poached eggs
2oz of Cheddar cheese	----or----	A baked potato with butter
A diary king cone of ice cream	----or----	1/4 lb. grilled rump steak
A medium sized grilled cod fillet	--or----	A dish of tinned fruit salad
Two poached eggs	----or----	A diary king cone of ice cream
A doughnut	----or----	A roast chicken breast
A large Cadbury's flake	----or----	2oz of Cheddar cheese

Appendix 1f: The *'free choice'* food questionnaire

Volunteer Number: ---------- Occasion Number: ---------

--

Date: ----------

Examine each individual item in turn to make your assessment. If you would like to eat it at this moment place a cross on the line next to it. If not, go on to the next. Consider each item independently from the others. Do not spend a long time on any item.

--------- A roast chicken breast
--------- A currant bun
--------- A large Cadbury's flake
--------- A medium sized peach
--------- A baked potato with a small knob of butter
--------- A medium sized grilled cod fillet
--------- Two average size tomatoes
--------- A grilled lean lamb cutlet
--------- A small size of cheesecake
--------- A small green salad
--------- A crusty white or brown bread roll
--------- Two slices of corned beef
--------- Four ginger biscuits
--------- A medium size sausage roll
--------- A dish of fresh strawberries
--------- Half a cup of tinned salmon
--------- Two pickled onions
--------- A small slice of jam-filled sponge cake
--------- A grilled lean piece of gammon
--------- Two lemon pancakes
--------- A medium sized dish of baked beans
--------- A carton of natural yoghurt
--------- One and a half packets of crisps
--------- A dish of shelled prawns
--------- A dish of tinned fruit salad
--------- A 2oz wedge of Cheddar cheese
--------- A 1/4 lb. grilled Rump steak
--------- Two sticks of celery
--------- A cream filled chocolate eclair
--------- A medium size bowl of rice
--------- A small slice of honeydew lemon

Appendix 2: Glossary of behavioural terms

Behaviour

Avoidance behaviour: Behaviour maintained by negative reinforcement; that is, responses prevent presentation of an aversive stimulus.

Escape behaviour: Behaviour maintained by negative reinforcement (see below) whereby responses terminate presentation of an aversive stimulus.

Conditioning

Classical conditioning: Procedures that present different stimuli in temporal proximity (***contiguity***) but where resulting responses have no reinforcing or aversive consequences (synonymous with ***Pavlovian*** conditioning).

Operant conditioning: A procedure whereby responses have defined reinforcing or aversive consequences that are instrumental in attainment of a goal. Contiguity may be a necessary condition in such procedures, but is not sufficient for the procedures to be described as instrumental.

Punisher

A stimulus that decreases the frequency of behaviour that leads to its presentation.

Stimulus

Stimulus: An environmental event that produces a change in the behaviour of an organism.

Aversive stimulus: A stimulus which causes the organism to behave so as to minimize exposure to it (as in negative reinforcement or punishment procedures). In evolutionary terms, aversive stimuli were probably developed to enable organisms to avoid noxious or harmful stimuli.

Discriminative stimulus: Cueing stimulus, indicating occasions when a response will be reinforced. It is also the effect which enables the organism to distinguish between drug and non-drug presentations.

Reinforcer

Positive reinforcer: A stimulus that increases the frequency of the behaviour that leads to its presentation. Drugs of abuse are strong positive reinforcers.

Primary reInforcer: A stimulus whose reinforcing effect appears to be inherent. This stimulus is described as ***unconditioned***, as it does not require learning or conditioning. Food and water are examples of primary reinforcers.

Secondary reinforcer: A stimulus whose reinforcing quality has been acquired via its association with a primary reinforcer. This is a ***conditioned*** stimulus.

Schedule of reinforcement: A programme which determines the relationship between responses and the occurrence of reinforcing stimuli. Under intermittent schedules, responses are reinforced occasionally, and specified numbers of responses at particular times may be required for reinforcement.

Second-order schedule of reinforcement: A complex schedule whereby primary reinforcement occurs only after completion of at least two consecutive components each of which terminates with presentation of a brief stimulus that has typically been paired with the primary reinforcer.

Response

The behavioural consequence of presenting a stimulus to an organism.

xxxx

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